Scientific Program

Sunday 2 December, 2012

10:00 – 12:00  Parkside G04
PGx: new research interests and directions workshop

14:30 – 16:30

<table>
<thead>
<tr>
<th>Chairs</th>
<th>Parkside 110A</th>
<th>Parkside G04</th>
<th>Parkside 110B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASCEPT-APSA Careers workshop</td>
<td>ASCEPT-APSA Clinical Pharmacology workshop</td>
<td>ASCEPT-APSA Education Forum workshop</td>
</tr>
<tr>
<td></td>
<td>Careers in the pharmaceutical industry</td>
<td>Introduction to TDM/TCI</td>
<td>Introduction and scene setting; TQSA; Standards Panel and TLOs</td>
</tr>
<tr>
<td></td>
<td>Dr Barbara Kemp-Harper, Ms Claire Johnston, Ms Alice Kane</td>
<td>Dr Matt Doogue</td>
<td>Prof Ieva Stupans, University of New England</td>
</tr>
<tr>
<td></td>
<td>Finding your way in the Pharmaceutical Industry</td>
<td>Measuring drug concentrations, analytical methods and issues</td>
<td>Mapping assessment to TLOs for pharmacy</td>
</tr>
<tr>
<td></td>
<td>Dr Annette Gross, GlaxoSmithKline R&amp;D</td>
<td>Dr Andrew Rowland</td>
<td>Prof Ieva Stupans, University of New England</td>
</tr>
<tr>
<td></td>
<td>It's a long way from rats and lab coats</td>
<td>Digoxin, a classic</td>
<td>What is the relevance of TLOs for the teaching of pharmacology?</td>
</tr>
<tr>
<td></td>
<td>Dr Richard Booth, iNOVA Pharmaceuticals</td>
<td>Prof Ray Morris</td>
<td>Dr Liz Davis, Monash University and Dr Tina Hinton, The University of Sydney</td>
</tr>
<tr>
<td></td>
<td>What I wish I knew about clinical trials roles and networking when I was sitting where you are today!</td>
<td>The perhexiline story</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr Craig Rayner, Roche</td>
<td>Prof John Horowitz</td>
<td></td>
</tr>
<tr>
<td></td>
<td>How to locate and maximise your success in finding your ideal job</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr Amanda Reid, Daryl Alexander &amp; Associates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

17:00 – 18:00  Parkside Auditorium
Welcome to country
Opening session and plenary 1
Prof David Le Couteur, ASCEPT President
Prof Andrew McLachlan, APSA President
Emeritus Prof Kim Oates AM, The University of Sydney

18:00 – 19:30 Welcome Reception
Parkside Foyer, Level 1

Monday 3 December, 2012

08:00 – 09:00  Parkside Foyer, Level 1
Poster presentations

09:00 – 10:00  Parkside Auditorium
Plenary 2: British Pharmacological Society lecture
Proton pump inhibitors - robust evidence of public health hazard, or just plain confounding? - 101
Dr Yoon Loke, The University of East Anglia, UK

10:00 – 10:30 Morning tea

10:30 – 12:30

<table>
<thead>
<tr>
<th>Chairs</th>
<th>Parkside Auditorium</th>
<th>Parkside 110A</th>
<th>Parkside 110B</th>
<th>Parkside G04</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symposium 1: Drug transporters in clinical pharmacokinetics and drug-drug interactions</td>
<td>Symposium 2: The yin and yang of novel receptor drug discovery paradigms</td>
<td>Emerging trends in dose individualisation in clinical practice</td>
<td>The patient journey</td>
</tr>
<tr>
<td></td>
<td>Dr Joseph Nicolazzo</td>
<td>Dr Meritxell Canals Buj</td>
<td>A/Prof Ross Norris</td>
<td>A/Prof Ines Krass</td>
</tr>
<tr>
<td></td>
<td>Transporter-based drug-drug interactions – principles and examples - 102</td>
<td></td>
<td>A/Prof Betty Sallustio</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prof Jashvant Unadkat, University of Washington, USA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Role of organic cation transporters in mediating oral drug absorption - 103</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr Oihren Thukker, University of North Carolina, USA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Role of transporters in mediating hepatabiliary clearance of drugs - 104</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prof Kim Brouwer, University of North Carolina, USA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Impact of BBB Transporters on Delivery and Efficacy of Molecularly-Targeted Agents in Brain Tumors - 105</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prof William Elmqvist, University of Minnesota, USA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflammation resolution mediators in tumour growth and metastasis: good, bad and indifferent - 106</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prof Alastair Stewart, The University of Melbourne</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exploring allosteric modulation and ligand-directed stimulus bias at the glucagon-like peptide-1 receptor - 107</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Denise Wootten, Monash University</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Physiological correlates of biased receptor signalling: relevance to opioid drug action in health and disease - 108</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr Laura Bohn, The Scripps Research Institute, Florida, USA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Revealing the potential of GPCR bias signalling - 109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prof Andrew Tobin, University of Leicester, UK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-parametric population PK models and their use in individual patient care - 110</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/Prof Michael Neely, University of Southern California, USA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Emerging trends in dose individualisation in clinical practice - 111</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/Prof Jennifer Martin, The University of Queensland</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pharmacogenomics in dose individualisation - 112</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prof Elizabeth Phillips, Institute for Immunology &amp; Infectious Diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dose individualisation of Dabigatrin - 113</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr Paul Chin, University of Otago, NZ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The patient journey towards the end of life-patient and family accounts of patient safety - 114</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ms Aileen Collier, Centre for Health Communication, University of Technology Sydney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Towards a medicinewise Australia: the NPS approach to improving health literacy - 115</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ms Karen Kaye, NPS Medicinewise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Location</td>
<td>Meetings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30 – 13:30</td>
<td>Parkside Foyer, Level 1</td>
<td>Lunch and poster presentations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside Auditorium</td>
<td>Pharmacogenomics SIG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside G04</td>
<td>New Zealand Forum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside 110B</td>
<td>Toxology and Drug Discovery SIG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside 110A</td>
<td>Neuro-behavioural SIG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30 – 15:30</td>
<td>Parkside 110B</td>
<td>Oral presentations 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside Auditorium</td>
<td>Oral presentations 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside G04</td>
<td>Oral presentations 3: Clinical Trainee</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside 110A</td>
<td>Oral presentations 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chairs</td>
<td>Dr Betty Exintaris</td>
<td>A/Prof Lynne Emmerton</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr Matt Doogue</td>
<td>Dr David Shackleford</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30 – 13:45</td>
<td></td>
<td>The effect of ageing on paracetamol pharmacokinetics and toxicity in Fischer 334 rats - 120 Mr John Mach, Kolling Institute of Medical Research, Royal North Shore Hospital, Sydney Medical School, The University of Sydney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>What parents want to know about attention-deficit hyperactivity disorder (ADHD): A qualitative investigation - 128 Miss Rana Ahmed, The Faculty of Pharmacy, The University of Sydney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Is the process for approval of high cost drugs for off-formulary use leading to clinically appropriate outcomes? - 128 Dr Catherine Lucas, Department of Clinical Pharmacology, Royal College of Physicians, Sydney Medical School, The University of Sydney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Differential effects of mango peel sub-fractions on lipid accumulation in 3T3-L1 adipocyte cells - 144 Mr Meng-Wong Taing, School of Pharmacy, The University of Queensland</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:45 – 14:00</td>
<td></td>
<td>NOX2B: A Novel Splice Variant of NOX2 That Promotes Reactive Oxygen Species (ROS) Production in Macrophages - 121 Dr Stavros Selenidis, Department of Pharmacology, Monash University</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The influence of disease and other factors on adherence - 129 Miss Piyan Assawasawannakit, School of Pharmacy, University of Otago, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The performance of the Cockcroft-Gault, MDRD and CKD-EPI equations in predicting gentamicin clearance - 137 Dr Paul Chin, Department of Clinical Pharmacology, Christchurch Hospital, NZ, Department of Medicine, University of Otago, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The preparation and characterisation of polypyrrole particles for tuneable drug delivery - 145 Mr Zaid Aryawe, The University of Auckland, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00 – 14:15</td>
<td></td>
<td>Bitter taste receptor agonists are novel bronchodilators of small airways in mouse lung slices - 122 Dr Jane Bourke, Dept of Pharmacology, University of Melbourne</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Strategies used to support patients’ adherence to medication: a national survey of community pharmacists - 130 Mrs Sarah Mansoor, Faculty of Pharmacy, The University of Sydney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Describing the use of antibiotics in acute exacerbations of chronic obstructive pulmonary disease - 138 Dr Mitchell McKean, Princess Alexandra Hospital</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Characterisation of emulsions as potential salvia substitutes in xerostomia - 146 Miss Sara Hanning, School of Pharmacy, University of Otago, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:15 – 14:30</td>
<td></td>
<td>Screening potential skin sensitizers using high throughput direct peptide activity assay - 123 Dr Sussan Ghassabian, Centre for Integrated Preclinical Drug Development, University of Queensland</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beyond expectations? Do pharmacists perform clinical interventions when carrying out advance support medication reviews? - 131 Dr Rhiannon Braund, School of Pharmacy, University of Otago, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytochrome P450 2C19 genotyping cost-effective for guiding clopidogrel treatment in Australia - 139 Dr Michael Sorich, Sansom Institute, University of South Australia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conformational stability of various proteins in solid lipid matrices prepared by melting and cooling - 147 Mr Farrukh Zeeshan, School of Pharmacy, University of Otago, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:30 – 14:45</td>
<td></td>
<td>The antimicrobial peptide LL-37 inhibits migration of the prostate cancer cells - 124 Ms Yan Tu, Department of Pharmacology, University of Melbourne</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The influence of disease and other factors on adherence - 129 Miss Piyan Assawasawannakit, School of Pharmacy, University of Otago, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The performance of the Cockcroft-Gault, MDRD and CKD-EPI equations in predicting gentamicin clearance - 137 Dr Paul Chin, Department of Clinical Pharmacology, Christchurch Hospital, NZ, Department of Medicine, University of Otago, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The preparation and characterisation of polypyrrole particles for tuneable drug delivery - 145 Mr Zaid Aryawe, The University of Auckland, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:45 – 15:00</td>
<td></td>
<td>Expression and localisation of Pannexin-1 Hemichannels in human colon in health and disease - 125 Miss Erica Diezmos, Dept of Pharmacology, School of Medical Sciences, University of New South Wales</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Effects of three different forms of inhaler technique education for health care professionals on patient asthma outcomes - 133 Dr Sinthia Bosnic-Anticevich, The University of Sydney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pro-migratory actions of prostacyclin in breast cancer cells that over-express cyclooxygenase-2 - 141 Dr Sarah Allison, Pharmacogenomics and Drug Development, Faculty of Pharmacy, The University of Sydney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The lymphatic system is critical in maintaining the prolonged circulation of monoclonal antibodies and in promoting absorption from the subcutaneous injection site - 149 Ms Annette Dahlberg, Monash Institute of Pharmaceutical Sciences, Monash University</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00 – 15:15</td>
<td></td>
<td>Neurokinin A potentiates spontaneous and purinergic evoked smooth muscle contraction and bladder afferent activity responses via activation of mouse bladder urothelial and detrusor NK2 receptors - 126 Mr Luke Grundy, Bond University</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Community pharmacy as a health hub: meeting the needs of people with chronic conditions - 134 Ms Sara McMillan, Griffith Health Institute, Griffith University</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A new pharmacokinetic abnormality among patients with 5-fluorouracil toxicity - 142 Dr John Duley, School of Pharm, PACE, University of Queensland, Mater Medical Research Institute</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stabilisation of amorphous indomethacin in aqueous suspensions: Effect of polymer addition method - 150 Dr Dorothy Saville, School of Pharmacy, University of Otago, NZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:15 – 15:30</td>
<td></td>
<td>Inflammatory biomarkers predict response and toxicity to FOLFIRI in colorectal cancer patients - 121 Miss Thi Em-Phuc Huynh, Discipline of Pharmacology, The University of Sydney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>“Knowing how” is not enough: why people with asthma do not maintain correct inhaler technique over time - 135 Ms Ludmila Ovchinikova, The Faculty of Pharmacy, The University of Sydney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-proliferative actions of sorafenib and its major metabolites in MDA-MB-231 breast cancer cells - 143 Dr Pei (Sarah) Cui, Faculty of Pharmacy, The University of Sydney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Understanding the role of device design on the aerosolisation of a carrier-based dry powder inhaler - 147 Qi Zhou, Advanced Drug Delivery Group, Faculty of Pharmacy, The University of Sydney</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
16:30 – 16:45
Contribution of rCtr1 to the uptake and toxicity of copper and platinum anticancer drugs in sensory neurons - 153
Dr Johnson Liu, School of Pharmacy, Faculty of Health Science, University of Tasmania, Dept of Pharmacol & Clin Pharmacol, FMHS, University of Auckland, NZ
The influence of phenotypic and genotypic patient characteristics on alloprolin and oxypyrulin pharmacokinetics in patients with gout - 161
Mr Daniel Trajkov, Faculty of Pharmacy, The University of Sydney
Renal function estimation in drug development: should East Asian ethnicity be considered? - 160
Mr Danial Trajkov, Faculty of Pharmacy, The University of Sydney
B-Alanine, A GABA partial agonist becomes antagonist when one residue at loop C of binding pocket mutated - 168
Mr Moewish Naffaa, Faculty of Pharmacy, The University of Sydney
The role of the secretory pathway calcium ATPases (SPCAs) in breast cancer - 176
Mrs Jane Lee, School of Pharmacy, The University of Queensland

16:15 – 16:30
Development and application of a mechanism-based model for the multistage life-cycle of murine malaria - the effect of single and multiple dose dihydroartemisinin - 159
Dr Kashyap Patel, Centre for Medicine Use and Safety, Monash University
Risk management plans in the Australian regulatory environment - 167
Miss Natalie Raffoul, Faculty of Pharmacy, The University of Sydney
Exploring cannabinoid receptor activity of synthetic compounds identified in “Spice”-related products using novel assays for CB1 and CB2 receptor activation - 175
Ms Jordyn Stuart, Australian School of Advanced Medicine, Macquarie University
Pharmacoregulation of distinct molecular phenotypes of the human CaSR caused by naturally occurring mutations - 183
Dr Katie Leach, Monash Institute of Pharmaceutical Sciences, Monash University

16:45 – 17:00
Association between intra-renal P-gp expression and cyclosporine concentrations in renal transplantation - 155
A/Prof Benedetta Sallustio, The Queen Elizabeth Hospital, The University of Adelaide/Adelaide Medical School
Gentamicin directed therapy: Which program to use? - 163
Mr Shaun Kumar, School of Medical Sciences, University of New South Wales, Department of Clinical Pharmacology, St Vincent's Hospital
The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin - 162
Dr Sophie Stocker, Dept of Bioengineering and Therapeutic Sciences, University of California San Francisco, USA
Potent anti-inflammatory effects of anaglographidine and its major metabolite, anaglographidine sulphonate - 171
Prof Gerald Muench, University of Western Sydney
Glucocorticoids inhibit breast tumour cell migration but increase metastasis to the lung in a mouse model of breast cancer - 178
Ms Ebony Fietz, The University of Melbourne

17:00 – 17:15
Population pharmacokinetic modelling of colistin methanesulphonate and formed colistin in patients on continuous ambulatory peritoneal dialysis - 156
Dr Cornelia Landersdorfer, Centre for Medicine Use and Safety, Monash University
Cleozapine-induced myocarditis: characterisation using case-control design - 164
Dr Kathlyn Ronaldson, Department of Epidemiology and Preventive Medicine, Monash University
Anaesthesia increases markers of inflammation and pro-inflammatory cytokines in serum and hippocampus: relationship to post-operative cognitive dysfunction - 172
Dr Jennifer Callaway, Pharmacology Department, University of Melbourne
Oleoyl-L-glycine and N-arachidonyl-glycine inhibit the glycine transporter GlyT2 - 179
Prof Robert Vandenberg, Discipline of Pharmacology, Bosch Institute, The University of Sydney

17:15 – 17:30
Development of a population model of early rheumatoid arthritis disease progression treated with methotrexate, sulfasalazine and hydroxychloroquine - 157
Ms Jessica Wojciechowski, Australian Centre for Pharmacometrics, University of South Australia
Relationship between high risk prescribing and adverse outcomes in people with and without Alzheimer's disease - 165
Dr Daniela Gnjidic, Faculty of Pharmacy, The University of Sydney, Clinical Pharmacology Department, Royal North Shore Hospital, Sydney Medical School, The University of Sydney
Hypoxia-inducible factor - 1 (HIF-1) prolyl hydroxylase inhibitors have neuroprotective actions in a neonatal rat model of hypoxic-ischemic brain injury - 173
Dr Nicole Jones, Department of Pharmacology, University of New South Wales
Delineating determinants of cooperativity, affinity and bias for mGlu5 allosteric modulators - 181
Dr Karen Gregory, Drug Discov Biol, MIPS, Monash Univ, Dept of Pharmacol & Ctr for Neurosci Drug Discov, Vanderbilt University Medical Centre, USA

17:30 – 17:45
Evaluation of current warfarin pharmacokinetic-pharmacodynamic models and dosing algorithms - 158
Miss Verty Pearson-Dennett, Australian Centre for Pharmacometrics, University of South Australia
A Collaboration to Measure ADEs in three District Health Boards - 166
Dr Chris Cameron, Department of Clinical Pharmacology, Capital & Coast DHB, NZ
Oxycodeone-induced activation of Toll-Like Receptor 4 contributes to drug reward - 174
Dr Mark Hutchinson, Discipline of Physiology, University of Adelaide
Nordihydroguaiaretic acid activates human TRPA1 - 182
Prof Mark Connor, Australian School of Advanced Medicine, Macquarie University

17:45 – 18:00
Development and application of a mechanism-based model for the multitarget life-cycle of murine malaria - the effect of single and multiple dose dihydroartemisinin - 159
Dr Kashyap Patel, Centre for Medicine Use and Safety, Monash University
Risk management plans in the Australian regulatory environment - 167
Miss Natalie Raffoul, Faculty of Pharmacy, The University of Sydney
Exploring cannabinoid receptor activity of synthetic compounds identified in “Spice”-related products using novel assays for CB1 and CB2 receptor activation - 175
Ms Jordyn Stuart, Australian School of Advanced Medicine, Macquarie University
Pharmacoregulation of distinct molecular phenotypes of the human CaSR caused by naturally occurring mutations - 183
Dr Katie Leach, Monash Institute of Pharmaceutical Sciences, Monash University

18:00
ASCEPT & APSA Presidents’ Function (by invitation only)

18:30 – 22:00
AAPS Student Dinner
Bungalow 8, 3 Lime Street, Sydney
<table>
<thead>
<tr>
<th>Time</th>
<th>Location</th>
<th>Meeting</th>
<th>SPONSORED BY</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00 – 09:00</td>
<td>Parkside Foyer, Level 1</td>
<td>Poster presentations</td>
<td></td>
</tr>
<tr>
<td>09:00 – 10:00</td>
<td>Parkside Auditorium</td>
<td>Plenary 3: APSA Lecture</td>
<td>SPONSORED BY NAPE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Newer drugs in older people: better the devil you know when it comes to medication safety? - 200 Prof Gregory Peterson, Professor of Pharmacy and Head of School, University of Tasmania</td>
<td></td>
</tr>
<tr>
<td>10:00 – 10:30</td>
<td></td>
<td>Morning tea</td>
<td></td>
</tr>
<tr>
<td>10:30 – 12:30</td>
<td>Parkside Auditorium</td>
<td>Symposium 5: Metabolites and medication safety</td>
<td>Flinders University</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Structure activity studies and therapeutic potential of venom peptides that target acid-sensing ion channels - 204 Dr Lachlan Rash, The University of Queensland</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Consequences of human TRPV4 mutations: implications for drug targeting and disease - 205 Prof Peter McIntyre, RMIT University</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GABA, receptors and flavonoids: Achieving subtype selectivity anxiolytics devoid of sedative effects - 206 Dr Mary Chebib, The University of Sydney</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRPM7 in cardiac hypertrophy: more than just a cation channel - 207 Dr Tamara Paravicini, The University of Queensland</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside 110A</td>
<td>Symposium 6: Ion channels in drug discovery: developing safer medications</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pharmacokinetic and pharmacodynamic modelling of PR-104: dissecting the “bystander effect” of hypoxia-activated metabolites - 202 Dr Kashyap Patel, Monash University</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metabolic drug activation in rheumatoid arthritis and drug toxicity - 203 Dr Michael Weise, University of South Australia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside 110B</td>
<td>Symposium 7: Drug safety-clinical pharmacology at the epicentre</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Systematic Reviews of Adverse Effects – Why Bother? - 208 Dr Yoon Loke, University of East Anglia, UK</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Public health: drug safety epidemiology - 209 Dr Kathlyn Ronaldson, Monash University</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pharmacogenomics of drug safety - 210 Prof Elizabeth Phillips, Murdoch University</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The safety of medicines in older people - 211 Prof David Le Couteur, The University of Sydney</td>
<td></td>
</tr>
<tr>
<td>12:30 – 13:30</td>
<td>Parkside Foyer, Level 1</td>
<td>Lunch and poster presentations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside 110A</td>
<td>Drug Disposition and Response SIG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside 110B</td>
<td>Education Forum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parkside Auditorium</td>
<td>Cardiovascular SIG</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Parkside Auditorium</td>
<td>Parkside G04</td>
<td>Parkside 110A</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Chairs</td>
<td>Dr Barbara Kemp-Harper</td>
<td>Prof Greg Peterson</td>
<td>Dr Sophie Stocker</td>
</tr>
<tr>
<td>13:30 – 13:45</td>
<td>Expression, regulation, dephosphorylation and putative nutrient-sensing function of taste GPCRs in the heart - 217</td>
<td>Safe to crush? Pilot study into solid dosage form modification in aged care - 225</td>
<td>High-throughput assay for simultaneous quantification of the plasma concentrations of morphine, fentanyl, midazolam and their major metabolites using automated SPE coupled to LC-MS/MS - 233</td>
</tr>
<tr>
<td></td>
<td>Mr Chakradhar Lagishetty, School of Biomedical Sciences, University of Queensland</td>
<td>Dr Greg Kyle, University of Canberra</td>
<td>Dr Sussan Ghassabian, Centre for Integrated Preclinical Drug Development, University of Queensland</td>
</tr>
<tr>
<td>13:45 – 14:00</td>
<td>A reduction in random between subject variability is not mandatory when adding a new covariate - 218</td>
<td>Can software assist the home medicines review process by identifying clinically relevant drug-related problems? - 226</td>
<td>Prescribing in the elderly - cytochrome P450 (CYP) enzyme inhibitors and substrates - 234</td>
</tr>
<tr>
<td></td>
<td>Miss Jacqueline Ku, Department of Pharmacology, Monash University</td>
<td>Mr Colin Curtain, School of Pharmacy, University of Otago, NZ</td>
<td>Dr Karen Kerr, School of Biomedical Sciences &amp; Pharmacy, University of Newcastle</td>
</tr>
<tr>
<td>14:00 – 14:15</td>
<td>Ghrelin gene-derived peptides have protective roles in the cerebral circulation and brain - 219</td>
<td>An observational study of pharmacists’ interventions to minimise medication misadventures in paediatric patients - 227</td>
<td>Fruit juices as perpetrators of drug interactions: The role of intestinal transporters - 235</td>
</tr>
<tr>
<td></td>
<td>Miss Teneale Stewart, School of Pharmacy, The University of Queensland</td>
<td>Ms Hesty Ramadaniati, School of Pharmacy, Curtin University</td>
<td>Mr Michael Bolton, Faculty of Pharmaceutical Sciences, University of Sydney</td>
</tr>
<tr>
<td>14:15 – 14:30</td>
<td>Calcium-dependent regulation of multidrug resistance-associated protein 3 (MRP2/ABCC3) gene expression in a model of epithelial-mesenchymal transition (EMT) - 220</td>
<td>Prevalence and factors associated with antidepressant use in Tasmanian nursing homes - 228</td>
<td>Ion chromatographic separation and isolation of oligosaccharides of intact low-molecular-weight heparin for the determination of their anticoagulant and anti-inflammatory properties - 236</td>
</tr>
<tr>
<td></td>
<td>Ms Teneale Stewart, School of Pharmacy, The University of Queensland</td>
<td>Ms Laura Trillini, Unit for Research and Education (UMORE) University of Tasmania</td>
<td>Mr Madhur Shastri, School of Pharmacy, University of Tasmania</td>
</tr>
<tr>
<td>14:30 – 14:45</td>
<td>Small airway reactivity to constrictors is differentially altered by chronic and acute inflammatory stimuli - 221</td>
<td>Prevalence of frailty and number of medicines use in elderly Australian: A comparison of four frailty measures - 229</td>
<td>The physicochemical stability of diluted iron polymaltose in polyvinyl chloride infusion bags - 237</td>
</tr>
<tr>
<td></td>
<td>Ms Chantel Donovan, Department of Pharmacology, University of Melbourne</td>
<td>Miss Imaina Widagdo, School of Pharmacy and Medical Sciences, University of South Australia</td>
<td>Dr Rahul Patel, School of Pharmacy, University of Tasmania</td>
</tr>
<tr>
<td>14:45 – 15:00</td>
<td>Characterisation of the hybrid orthosteric/allosteric bitopic nature of TBPB at the M1 muscarinic ACh receptor - 222</td>
<td>Pharmacy staff perspectives on patient safety issues involved in the supply of dose administration aids - 230</td>
<td>The physicochemical stability of Ceftazidime or Cephazolin in peritoneal dialysis freisenius infusion bag - 238</td>
</tr>
<tr>
<td></td>
<td>Mr Peter Keov, Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University</td>
<td>Mr Ramesh Wairpola, Faculty of Pharmacy, The University of Sydney</td>
<td>Miss Siti Farahwahida Shikh Mohd Fazdilah, School of Pharmacy, University of Tasmania</td>
</tr>
<tr>
<td>15:00 – 15:15</td>
<td>CYP2B6*6 mutation significantly reduces in vitro N-demethylation of ketamine enamtoners - 223</td>
<td>Key health professionals’ views of prescribing resources for older patients - 231</td>
<td>A practical synthesis of D-rhamnosyl building blocks for synthetic bacterial O-polyasaccharide conjugate vaccines - 239</td>
</tr>
<tr>
<td></td>
<td>Mr Yibai Li, Discipline of Pharmacology, University of Adelaide</td>
<td>Ms Paulina Stehlik, Centre for Medication Use and Safety, Monash University</td>
<td>Mr Matthew Zunk, School of Pharmacy, Griffith University, Institute for Glycomics, Griffith University</td>
</tr>
<tr>
<td>15:15 – 15:30</td>
<td>Simultaneous deletion of the α1- adrenoceptor and P2X2, purinoreceptors generate male mice which are infertile - 224</td>
<td>Major medication discrepancies in patients with type 2 diabetes mellitus (T2DM), referred from primary care, to a tertiary ambulatory diabetes centre - 232</td>
<td>Glutamate efflux from brain to blood - transport and uptake studies in a bovine in vitro blood-brain barrier model - 240</td>
</tr>
<tr>
<td></td>
<td>Mr Carl White, Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University</td>
<td>Miss Matronna Fiona Azzi, Faculty of Pharmacy - The University of Sydney</td>
<td>Dr Birger Brodin, Department of Pharmacy, University of Copenhagen, Denmark</td>
</tr>
<tr>
<td>15:30 – 16:00</td>
<td>Afternoon tea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00 – 17:00</td>
<td>Plenary 4: ASPECT Lecture Much ado about NSAIDs - 249</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prof Kathie Knights, Department of Clinical Pharmacology, Flinders University</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00 – 18:00</td>
<td>Parkside 110A</td>
<td>Parkside Auditorium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>APSA AGM</td>
<td>ASPECT AGM</td>
<td></td>
</tr>
<tr>
<td>19:00 – 22:30</td>
<td>Conference Dinner Dockside, The Balcony Level, Cockle Bay Wharf, Darling Harbour</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Wednesday 5 December, 2012**

<table>
<thead>
<tr>
<th>Time</th>
<th>Location</th>
<th>Session Title</th>
<th>Chairs</th>
<th>Presenters</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00 – 10:00</td>
<td>Parkside Auditorium</td>
<td>APSA Medal Oration 2012</td>
<td>Prof Andrew Gilbert, University of South Australia</td>
<td></td>
</tr>
<tr>
<td>10:00 – 10:30</td>
<td>Morning tea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30 – 12:30</td>
<td>Parkside Auditorium</td>
<td>Symposia 9: Joint ASCET-APSA education symposium</td>
<td>Dr Liz Davis</td>
<td>Myocardial stress and ischemic injury - sex and sex steroid influences - 304</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prof Lea Delbridge, The University of Melbourne</td>
<td>Prof Lea Delbridge, The University of Melbourne</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dr Richard Loiacono, Monash University</td>
<td>Y chromosome dependent blood pressure regulation in the SHRSP is mediated, in part</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>by the renal reninangiotensin system - 305</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dr Amanda Sampson, Baker IDI Heart and Diabetes Institute</td>
<td>Sex differences in the role of the renin-angiotensin system in the regulation of</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>arterial pressure and renal function - 306</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A/Prof Kate Denton, Monash University</td>
<td>A/Prof Anthony Hannan, University of Melbourne</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Association of the human Y chromosome and cardiovascular disease risk - 308</td>
</tr>
<tr>
<td>12:30 – 13:30</td>
<td>Parkside Foyer, Level 1</td>
<td>Lunch and poster presentations</td>
<td></td>
<td>Systems based approach to drug safety - 309</td>
</tr>
<tr>
<td>13:30 – 15:30</td>
<td>Parkside Auditorium</td>
<td>Oral presentations 13</td>
<td>Prof David Le Couture</td>
<td>Dr Romano Fois, The University of Sydney</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dr Liz Davis</td>
<td>CareTrack as a methodology and relevance for med safety - 310</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A/Prof Simon Bell</td>
<td>Prof Ric Day, St Vincent's Hospital</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Injectable and Oral Contraceptive Use and Cancers of the Breast, Cervix, Ovary, and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Endometrium in Black South African Women: Case-Control Study - 311</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dr Emily Banks, Australian National University</td>
</tr>
<tr>
<td>13:45 – 14:00</td>
<td>Parkside Auditorium</td>
<td>Online support for pharmacology practical teaching - 317</td>
<td>Prof David Dwhurst, University of Edinburgh and British Pharmacological</td>
<td>Safety before efficacy? Australian pharmacists’ attitude to homeopathic products in pharmacy - 324</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Society, University of Edinburgh, UK</td>
<td>Ms Suzanne Schultz, School of Pharmacy and Medical Sciences, University of South Australia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ms Rachel George, The University of Sydney</td>
</tr>
<tr>
<td>14:00 – 14:15</td>
<td>Parkside Auditorium</td>
<td>Preventing resistance of bacterial “superbugs” by synergistic combinations of</td>
<td>Dr Stephanie Hennig, The University of Queensland</td>
<td>Exploring community pharmacists’ views on medication-safety management - 325</td>
</tr>
<tr>
<td></td>
<td></td>
<td>available antibiotics - 313</td>
<td>Dr Jurgen Builta, Monash University</td>
<td>Ms Rachel George, The University of Sydney</td>
</tr>
<tr>
<td>14:15 – 14:30</td>
<td>Parkside Auditorium</td>
<td>Development of an experiential and learning programme for undergraduate</td>
<td>Dr Alexandra Bennett, NSW Therapeutic Advisory Group</td>
<td>Improving consumer access to medicines: innovative medicines reclassification in New</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pharmacy students at Alcohol and Drug Services - 318</td>
<td></td>
<td>Zealand (NZ) - 326</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dr Juanita Westbury, Unit for Medication Outcomes, Research and Education</td>
<td>Ms Natalie Gould, University of Auckland, NZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(UMORE), University of Tasmania</td>
<td></td>
</tr>
<tr>
<td>14:30 – 14:45</td>
<td>Parkside Auditorium</td>
<td>Experimental design and statistical analysis in intermediate medical sciences</td>
<td>Dr Tina Hinton, School of Medical Sciences (Pharmacology), The University of Sydney</td>
<td>Community-acquired pneumonia: why aren’t national antibiotic guidelines followed? - 327</td>
</tr>
<tr>
<td></td>
<td></td>
<td>curricula - a pilot study - 321</td>
<td></td>
<td>Mr Maher Almatar, University of Tasmania</td>
</tr>
<tr>
<td>14:45 – 15:00</td>
<td>Parkside Auditorium</td>
<td>Effect of an educational workshop on pharmacists’ knowledge, attitudes and</td>
<td>Miss Christina Abdel Shaheed, Faculty of Pharmacy, The University of</td>
<td>Implications of the Personally-Controlled Electronic Health Record for community pharmacy - 329</td>
</tr>
<tr>
<td></td>
<td></td>
<td>beliefs towards low back pain (LBP) - 316</td>
<td>Sydney</td>
<td>A/Prof Lynne Emmerton, School of Pharmacy, CHIRI, Curtin University</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dr Celine Valant, Drug Discovery Biology</td>
<td></td>
</tr>
<tr>
<td>15:00 – 15:15</td>
<td>Parkside Auditorium</td>
<td>Use of an audience response system and collaborative learning increases</td>
<td>Dr Slade Matthews, Pharmacology, Sydney Medical School, The University</td>
<td>Challenges and safety concerns for community pharmacy personnel in the provision of services</td>
</tr>
<tr>
<td></td>
<td></td>
<td>engagement in post graduate pharmacy - 323</td>
<td>of young people - 329</td>
<td>to young people - 329</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mrs Amyna Helou, Centre for Medicine Use and Safety, Monash University</td>
<td>Ms Emma Horsfield, School of Pharmacy, The University of Auckland, NZ</td>
</tr>
<tr>
<td>15:15 – 15:30</td>
<td>Parkside Auditorium</td>
<td>Management of high blood pressure in pregnant women attending an Australian</td>
<td>Mrs Amyna Helou, Centre for Medicine Use and Safety, Monash University</td>
<td>Management of high blood pressure in pregnant women attending an Australian</td>
</tr>
<tr>
<td></td>
<td></td>
<td>maternity hospital - 331</td>
<td></td>
<td>maternity hospital - 331</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30 – 16:00</td>
<td>Parkside Auditorium</td>
<td>Awards and close</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Monday 3 December, 8.00am – 9.00am and 12.30pm – 1.30pm

PARKSIDE FOYER, LEVEL 1

<table>
<thead>
<tr>
<th>Board number</th>
<th>Abstract title</th>
<th>Presenter</th>
<th>Paper reference</th>
<th>Session theme</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>Pharmacovigilance and computational chemistry approaches to predicting torsade de pointes</td>
<td>Christabel Abalao</td>
<td>400</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>401</td>
<td>Tocomin restores endothelium-dependent relaxation in diabetic rat aorta</td>
<td>Saher Ali</td>
<td>401</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>402</td>
<td>Resistin, a novel adipokine, increases renal SNA and reduces BAT SNA using PI3-kinase or ERK 1/2 mediated mechanisms</td>
<td>Emilio Badner</td>
<td>402</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>403</td>
<td>Head-to-head comparison of the relative anti-fibrotic efficacy of H2 relaxin toa clinically used ACE inhibitor (enalapril)</td>
<td>Hasangkra Bodaragama</td>
<td>403</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>404</td>
<td>Comparing the anti-fibrotic actions of relaxin vs an angiotensin AT1 receptor blocker and AT2 receptor agonist</td>
<td>Jacqueline Chew</td>
<td>404</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>405</td>
<td>Is insulin-mediated sensitization of platelets to NO in diabetes TxA2P-dependent?</td>
<td>Cher-Rin Chong</td>
<td>405</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>406</td>
<td>Utilisation of anti-thrombotics for secondary stroke prevention at hospital discharge</td>
<td>Ashraf Eissa</td>
<td>406</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>407</td>
<td>Blood pressure lowering therapy for stroke patients at hospital discharge</td>
<td>Ashraf Eissa</td>
<td>407</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>408</td>
<td>AT2 and Mas receptor agonists as neuroprotective drugs for stroke</td>
<td>Megan Evans</td>
<td>408</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>409</td>
<td>Compound 21, a synthetic AT2 receptor agonist, evokes neuroprotection in a conscious rat model of ischaemic stroke</td>
<td>Lachlan Facey</td>
<td>428</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>410</td>
<td>Expression, regulation, dephosphorylation and putative nutrient-sensing function of taste GPCRs in the heart</td>
<td>Simon Foster</td>
<td>417</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>411</td>
<td>Capsaicin relaxes arteries via NO-dependent and independent mechanisms</td>
<td>Kirsty Fuller</td>
<td>411</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>412</td>
<td>Vanilloid-like agents inhibit platelet aggregation in vitro</td>
<td>Dom Geraghty</td>
<td>412</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>413</td>
<td>Psychotropic drugs inhibit the acetylcholine receptor-operated potassium current (IK.ACh) by different mechanisms in guinea-pig atrial myocytes</td>
<td>Yukio Hara</td>
<td>413</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>414</td>
<td>Effect of diabetes on the production and vasoactivity of hydrogen sulfide in rat middle cerebral arteries</td>
<td>Joanne Hart</td>
<td>414</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>415</td>
<td>Contractile recovery induced by pharmacological conditioning with GTN &amp; cariporide at cardioplegia</td>
<td>Mark Hicks</td>
<td>415</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>416</td>
<td>Relaxation of rat isolated pulmonary arteries by a novel vasodilator, BAY41-8543</td>
<td>Richard Hughes</td>
<td>416</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>417</td>
<td>Heterogeneous vasoconstrictor responses amongst men and women</td>
<td>Amenah Jachou</td>
<td>241</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>418</td>
<td>Pharmacological profiling of angiotensin II and bradykinin receptor heteromers</td>
<td>Elizabeth Johnston</td>
<td>418</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>419</td>
<td>Region-dependent contribution of the T-type voltage-operated calcium channel to rat renal vascular contraction</td>
<td>Makhal A Khannay</td>
<td>419</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>420</td>
<td>Brain infarct volume after permanent focal ischemia is not dependent on Nox2 expression</td>
<td>Hyun Ah Kim</td>
<td>420</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>421</td>
<td>Ghrelin gene-derived peptides have protective roles in the cerebral circulation and brain</td>
<td>Jacqueline Ku</td>
<td>219</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>422</td>
<td>Neuroprotective Effect of an Angiotensin Receptor-2 Agonist Following Cerebral Ischemia In Vivo and In Vivo</td>
<td>Seyeong Lee</td>
<td>422</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>423</td>
<td>The role of NOX2 NADPH oxidase in macrophage polarisation</td>
<td>Caitlin Lewis</td>
<td>242</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>424</td>
<td>Endothelin and endothelin receptor antagonism in rat isolated cerebral arteries:relevance for subarachnoid haemorrhage</td>
<td>Yohannes Mamo</td>
<td>424</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>425</td>
<td>Chronic NaHS treatment protects vascular function by reducing oxidative stress in streptozotocin-induced diabetes in mice</td>
<td>Hooi Hooi Ng</td>
<td>425</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>426</td>
<td>Review of epidemiology and management of atrial fibrillation in developing countries</td>
<td>Tu Nguyen</td>
<td>426</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>427</td>
<td>An allosteric enhancer of the adenosine A1 receptor improves cardiac function following ischaemia in isolated murine hearts</td>
<td>Roselyn Rose/Meyer</td>
<td>427</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>428</td>
<td>The DPP-4 inhibitor linagliptin improves endothelium-dependent relaxation of rat mesenteric arteries in the presence of high glucose</td>
<td>Salheen Salheen</td>
<td>428</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>429</td>
<td>Direct stimulation of AT2R and the MasR prevents TNFα-induced endothelial inflammation</td>
<td>Amanda Sampson</td>
<td>429</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>430</td>
<td>Review of antithrombotic risk assessment tools for stroke prevention in atrial fibrillation patients</td>
<td>Yishen Wang</td>
<td>430</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>465</td>
<td>Medication safety issues in older Australians: results from a national medicines census</td>
<td>Jo Barnes</td>
<td>465</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>466</td>
<td>Study of natural health product adverse reactions (SONAR): active surveillance of adverse events following concurrent natural health product and prescription medicine use in community pharmacies</td>
<td>Jo Barnes</td>
<td>466</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>467</td>
<td>The impact of medications on robust and frail older people with falls</td>
<td>Sarah Hlatner</td>
<td>467</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>468</td>
<td>An essential medicines list for children: Are they still therapeutic orphans?</td>
<td>Noel Cranwick</td>
<td>468</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>469</td>
<td>The ABCD of Clinical Pharmacokinetics</td>
<td>Matthew Dougue</td>
<td>469</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>470</td>
<td>A population pharmacokinetic (popPK) model to describe the renal clearance and the effect of transporter genetic variants on the pharmacokinetics of metformin in patients with type 2 diabetes mellitus (T2DM)</td>
<td>Jannie Duong</td>
<td>470</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>471</td>
<td>Population pharmacokinetics of factor VIII in paediatric patients</td>
<td>Kirsten Jensen</td>
<td>471</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>472</td>
<td>The pharmacokinetics of paracetamol and its metabolites: A population approach to investigating hepatic intrinsic clearance in old age</td>
<td>Claire Johnston</td>
<td>472</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>473</td>
<td>Baseline plasma urate: the only determinant for dosage selection of allopurinol</td>
<td>Diluk Kannaingara</td>
<td>473</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>474</td>
<td>Optimising ribavirin therapy for hepatitis C patients</td>
<td>Ashmit Kaur</td>
<td>474</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>475</td>
<td>High dose intravenous paracetamol in major surgical patients</td>
<td>Julia Kennedy</td>
<td>475</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>476</td>
<td>Dosage adjustment of medications in patients with renal impairment: how consistent are drug information sources?</td>
<td>Aarati Khanal</td>
<td>476</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>477</td>
<td>Development and validation of software to calculate the drug burden index: a pilot study</td>
<td>Lisa Koualadjian</td>
<td>477</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>478</td>
<td>Gentamicin directed therapy: Which program to use?</td>
<td>Shaan Kurniati</td>
<td>163</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>479</td>
<td>Identifying effective strategies to prevent drug-drug interactions in hospital: a user-centered approach</td>
<td>Olivia Misiakos</td>
<td>479</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>480</td>
<td>Ropivacaine concentrations (total and unbound) following transverse abdominis plane (TAP) block for analgesia following abdominal surgery - A pilot study</td>
<td>Raymond G Morris</td>
<td>480</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>Board number</td>
<td>Abstract title</td>
<td>Presenter</td>
<td>Paper reference</td>
<td>Session theme</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-------------------------------</td>
<td>-----------------</td>
<td>--------------</td>
</tr>
<tr>
<td>481</td>
<td>Impact of pharmacist-led dosing of vancomycin and aminoglycosides in hospitalised patients</td>
<td>Daniel O’Brien</td>
<td>481</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>482</td>
<td>Development of a population PKPD model to describe the effect of paracetamol on the International Normalised Ratio (INR)</td>
<td>Katie H Owens</td>
<td>482</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>483</td>
<td>Assessment of frailty and prescribing criteria in older people: A systematic review</td>
<td>Arlyn Poudel</td>
<td>483</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>484</td>
<td>Risk management plans in the Australian regulatory environment</td>
<td>Natalie Raffoul</td>
<td>167</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>485</td>
<td>Alliporinol Hypersensitivity (AH): An examination of all published cases, 1950-2011</td>
<td>Sheena Ramasamy</td>
<td>485</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>486</td>
<td>Association between age, lean body weight, frailty and inducible SIRT1 expression response to sepsis: the CHAMP study</td>
<td>Shajjia Razi</td>
<td>486</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>487</td>
<td>An evaluation of switches in therapeutic equivalents as triggers for adverse event monitoring</td>
<td>David Reith</td>
<td>487</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>488</td>
<td>Measuring drug concentrations, analytical methods and issues</td>
<td>Andrew Rowland</td>
<td>488</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>489</td>
<td>New Zealand Formulary - a case study of national formulary development</td>
<td>Bryan Simpson</td>
<td>489</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>490</td>
<td>Evaluation of formulary applications in Australian paediatric hospitals</td>
<td>Yashwant Sinha</td>
<td>490</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>491</td>
<td>Diagnostic errors in older patients: A systematic review of the incidence and causes in thirteen prevalent conditions</td>
<td>Jennifer Martin</td>
<td>491</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>492</td>
<td>An evaluation of a change in dosing regimen of gentamicin in neonates</td>
<td>Louise Thomas</td>
<td>492</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>493</td>
<td>Anti-arthritic and anti-protease activities of colloidal metallic silver (CMS)</td>
<td>Michael Whitehouse</td>
<td>493</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>494</td>
<td>Monitoring the anti-protease activity of colloidal metallic silver (CMS) in vivosing the Metatron/ Hunter</td>
<td>Michael Whitehouse</td>
<td>494</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>495</td>
<td>Posterior, subcapsular cataract in association with fluoroconisone acetatetherapy: a case report</td>
<td>Iain Whyte</td>
<td>495</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>496</td>
<td>Evaluating posaconazole use in a patient population</td>
<td>William Wu</td>
<td>496</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>501</td>
<td>Electronic prescribing in St Vincent’s hospital and the National Inpatient Medication Chart (NIMC) audit</td>
<td>Brian Egan</td>
<td>501</td>
<td>Clinical Pharmacology, Pharmacy Practice Training</td>
</tr>
<tr>
<td>517</td>
<td>A rapid and sensitive LC-MS method for the quantification of nitilotinib in human plasma</td>
<td>Daniel Barratt</td>
<td>517</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>518</td>
<td>Transplacental disposition of rosiglitazone in the maternal fetal unit</td>
<td>Maryam Bazargan</td>
<td>518</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>519</td>
<td>Glucosidation and glucuronidation of mycophenolic acid (MPA) by UDP-glucuronosyltransferase (UGT) 1A and 2B sub-family enzymes</td>
<td>Nuy Chau</td>
<td>519</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>520</td>
<td>Mega-model of voriconazole population pharmacokinetics</td>
<td>Michael Dolton</td>
<td>520</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>521</td>
<td>Cediranib and erlotinib are potent inhibitors of human solute carrier transporters</td>
<td>Rosie Johnston</td>
<td>521</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>522</td>
<td>Liver fibrosis as the major determinant of the altered hepatic uptake of taurolactone in rat model of liver diseases</td>
<td>Peng Li</td>
<td>522</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>523</td>
<td>In vivo bio-distribution of water-dispersible CdtE/CdtS quantum dots following intrahepatic injection</td>
<td>Xiaowen Liang</td>
<td>523</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>524</td>
<td>Intravital imaging of fluorescein transport in the rat liver after intrahepatic injection</td>
<td>Xin Liu</td>
<td>524</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>525</td>
<td>Effect of liver endothelial cell defenestration on hepatic insulin and glucose uptake</td>
<td>Victoria Cogger</td>
<td>525</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>526</td>
<td>Diurnal variation of CYP1A2 Activity in individuals of South Asian and European Ancestry</td>
<td>Vidyta Perera</td>
<td>526</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>527</td>
<td>Inhibition of human UDP-Glucuronosyltransferase 1A (UGT1A) enzymes by three different Tyrosine Kinase Inhibitors (Lapatinib, Pazopanib and Sorafenib): implications for drug interactions and jaundice</td>
<td>Andrew Rowland</td>
<td>527</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>528</td>
<td>In vitro characterisation of the ‘albumin effect’ on human liver microsomallanzapine oxidative metabolism</td>
<td>Ponnita Korprasethaworn</td>
<td>528</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>529</td>
<td>Allometric scaling of antimalarial drugs</td>
<td>Gang A Senaratna</td>
<td>529</td>
<td>Drug Disposition and Response</td>
</tr>
<tr>
<td>537</td>
<td>Effectiveness of a blended learning approach in delivering advanced drug delivery systems to third year pharmacy students at Curtin University</td>
<td>Yan Chen</td>
<td>537</td>
<td>Education</td>
</tr>
<tr>
<td>538</td>
<td>Evaluation as best practice: a case study on interdisciplinary learning and teaching of a Remote Health Experience program at Flinders NT</td>
<td>Pascale Dettwiler</td>
<td>538</td>
<td>Education</td>
</tr>
<tr>
<td>539</td>
<td>The eBook ‘Pharmacology in One Semester’: uses and student evaluation</td>
<td>Sheila Doggrell</td>
<td>539</td>
<td>Education</td>
</tr>
<tr>
<td>540</td>
<td>Quality assurance exercise for assessing basic compounding skills of pharmacy students</td>
<td>Jennifer Dobuzelli</td>
<td>540</td>
<td>Education</td>
</tr>
<tr>
<td>541</td>
<td>What really makes students ‘work ready’ - what are Pharmacy students’ and their preceptors’ considerations?</td>
<td>Jasmina Feping</td>
<td>541</td>
<td>Education</td>
</tr>
<tr>
<td>542</td>
<td>Bringing competency standards to life for UTAS Bachelor of Pharmacy Undergraduate students</td>
<td>Rose Nash</td>
<td>542</td>
<td>Education</td>
</tr>
<tr>
<td>543</td>
<td>The UTAS Pharmacy Students’ Road Map</td>
<td>Rose Nash</td>
<td>543</td>
<td>Education</td>
</tr>
<tr>
<td>544</td>
<td>Competency of Pharmacy graduates-tool validation and investigation</td>
<td>Joy Spark</td>
<td>544</td>
<td>Education</td>
</tr>
<tr>
<td>545</td>
<td>Jack and the beanstalk, climbing the vine of skills - preceptor training for pharmacists</td>
<td>Catherine Spiller</td>
<td>545</td>
<td>Education</td>
</tr>
<tr>
<td>546</td>
<td>Supporting Tasmanian hospital pharmacists to mentor pharmacy students</td>
<td>Catherine Spiller</td>
<td>546</td>
<td>Education</td>
</tr>
<tr>
<td>547</td>
<td>Fostering deep learning through collaborative learning and peer assessment: A case study in Pharmacology Teaching and Learning</td>
<td>Lisa Tee</td>
<td>547</td>
<td>Education</td>
</tr>
<tr>
<td>548</td>
<td>Adding creativity to pharmacy practice: Using Bloom’s Taxonomy to develop learning skills in fourth year pharmacy students</td>
<td>Hung Tran</td>
<td>548</td>
<td>Education</td>
</tr>
<tr>
<td>593</td>
<td>Computational Modeling of Powder Dispersions in Turbuhaler®</td>
<td>Qi (Tony) Zhou</td>
<td>593</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>594</td>
<td>Elucidating drug release mechanisms of biomacromolecule-containing lipid particles during lipolysis</td>
<td>Philip Christophersen</td>
<td>594</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>595</td>
<td>A comparison study on the effect of dry and moist heat sterilisation on lecithin microemulsions</td>
<td>Khubad Alihara</td>
<td>595</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>596</td>
<td>UV imaging and flow through Raman spectroscopy: information-rich tools for characterising the dissolution behaviour of furosemide</td>
<td>Sarah Gordon</td>
<td>596</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>597</td>
<td>Higher apparent solubility and faster dissolution rate of amorphous furosemide salt leads to faster Tmax after oral dosing in rats compared to amorphous and crystalline furosemide acid</td>
<td>Line Hagner Nielsen</td>
<td>597</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>598</td>
<td>Addition of hydroxypropyl methylcellulose to furosemide increases physical stability of the amorphous form of furosemide</td>
<td>Line Hagner Nielsen</td>
<td>598</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>Board number</td>
<td>Abstract title</td>
<td>Presenter</td>
<td>Paper reference</td>
<td>Session theme</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------------------------</td>
<td>-----------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>599</td>
<td>Comparison of two extraction methods prior to chromatographic determination of metabolic fragments of beta-endorphin within inflamed rat tissue</td>
<td>Naghmeh Asvadi</td>
<td>599</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>600</td>
<td>Polarized light microscopy as a method for analyzing API precipitation in simulated gastric media</td>
<td>Linda Jensen</td>
<td>600</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>602</td>
<td>Nicotine retention as the major determinant of percutaneous absorption of nicotine</td>
<td>Rina Kuswanyuning</td>
<td>602</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>603</td>
<td>Oral delivery of nanoparticles to the colon for tumour targeting</td>
<td>Yiming Ma</td>
<td>603</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>604</td>
<td>Implications of the use of thickening agents to aid swallowing of altered medicines: in vitro study</td>
<td>Yady Juliana Marinique-Tomes</td>
<td>604</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>605</td>
<td>A mechanistic based approach for enhancing buccal mucosalhesion of chitosan</td>
<td>Emil Meng-Lund</td>
<td>605</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>606</td>
<td>Validation of Microtitre plate assay for Haemophilus influenzae spp. Biofilm</td>
<td>Najia Obaid</td>
<td>606</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>607</td>
<td>Stability of key antioxidant compounds in poha (Sonchus oleraceus L.) leaf extracts under different post-harvest processes and storage conditions</td>
<td>Zong-Quan Ou</td>
<td>607</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>608</td>
<td>Optimization of simulated gastric media (FaSSGF) based on rheological characterization of human gastric fluid</td>
<td>Pernille Barbre Pedersen</td>
<td>608</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>609</td>
<td>The effect of haemocrit on insulin levels from dried blood spots for use in clinical studies</td>
<td>Emma Saills</td>
<td>609</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>610</td>
<td>The effect of formulation on the penetration of coated and uncoated zinc oxide nanoparticles into the viable epidermis of human skin in vivo</td>
<td>Washington Sanchez</td>
<td>610</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>611</td>
<td>Allosteric modulation of regulatory protein recruitment to the glucagon-like peptide-1 receptor</td>
<td>Emilija Savage</td>
<td>611</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>612</td>
<td>Targeting γ-alanyl aminopeptidase in Pseudomonas aeruginosa</td>
<td>Mohamed Sharjaki</td>
<td>612</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>613</td>
<td>Variability of anti-inflammatory diterpenoids from the Northern Kaanju medicinal plant, Dodonaea polyandra</td>
<td>Bradley Simpson</td>
<td>613</td>
<td>Pharmaceutical Science</td>
</tr>
<tr>
<td>620</td>
<td>Characterisation of spontaneous activity in the human prostate gland</td>
<td>Basu Chakrabarty</td>
<td>620</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>621</td>
<td>Effects of stinging nettle leaf extract on smooth muscle contractility in the isolated rat prostate gland</td>
<td>Nicole Eise</td>
<td>621</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>622</td>
<td>Depressed contractile responses of the bladder detrusor and urethral/lamina propria following luminal administration of a cytotoxic agent gemcitabine</td>
<td>Stefanie Farr</td>
<td>622</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>623</td>
<td>Distribution of 5-HT receptors and interacting proteins in human colonic tissue layers</td>
<td>Helen Irving</td>
<td>623</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>624</td>
<td>Mitomycin C alters urethral ATP, acetylcholine and PGE2 release in vitro</td>
<td>Sung-Hyun Kang</td>
<td>624</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>625</td>
<td>Effects of acrolein, a metabolite of Cyclophosphamide and Ifosfamide, on cultured human urothelial cells</td>
<td>Kyle Mills</td>
<td>625</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>626</td>
<td>P-glycoprotein expression level in treatment - resistant Helicobacter pylori patients</td>
<td>Marhanis Salihah Omar</td>
<td>626</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>627</td>
<td>Nerve-evoked and phasic contractions of the rat bladder: effects of low testosterone and treatment with the selective androgen receptor modulator trenbolone</td>
<td>Donna Sellers</td>
<td>627</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>628</td>
<td>Low prevalence of Helicobacter pylori infection among patients with Ulcerativecolitis in Ukraine</td>
<td>Tetyana Ternushchak</td>
<td>628</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>629</td>
<td>Simultaneous deletion of the α1A-adrenoceptor and P2X1 purinoceptor generates male mice which are infertile</td>
<td>Carl White</td>
<td>224</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
<tr>
<td>630</td>
<td>Complexity in 5-HT3 receptors: An optimized expression system for electrophysiology studies</td>
<td>Nor Syafinaz Yaskob</td>
<td>630</td>
<td>Urogenital and Gastrointestinal</td>
</tr>
</tbody>
</table>

Tuesday 4 December, 8.00am – 9.00am and 12.30pm – 1.30pm
PARKSIDE FOYER, LEVEL 1

<table>
<thead>
<tr>
<th>Board number</th>
<th>Abstract title</th>
<th>Presenter</th>
<th>Paper reference</th>
<th>Session theme</th>
</tr>
</thead>
<tbody>
<tr>
<td>432</td>
<td>Allostereic modulation of a chemogenetically modified G-protein-coupled receptor</td>
<td>Aila Abdul-Ridha</td>
<td>432</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>433</td>
<td>The effect of morphine on the growth and dissemination of breast tumor in mice</td>
<td>Marie-Odile Parat</td>
<td>433</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>434</td>
<td>Altered purinergic receptor calcium signalling associated with hypoxia in MDA-MB-468 breast cancer cells</td>
<td>Imran Azizim</td>
<td>434</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>435</td>
<td>Functional selectivity at the Adenosine A1 receptor: Implications for cytoprotection</td>
<td>Jo-Anne Baltos</td>
<td>435</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>436</td>
<td>Can interference of GIP activity modulate GPCRs for therapeutic gains?</td>
<td>Kenneth Chinkwo</td>
<td>436</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>437</td>
<td>Activation and acute desensitization of μ-opioid receptor wild-type and mutants with deleted phosphorylation sites</td>
<td>Marina Santiago</td>
<td>437</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>438</td>
<td>Selective GPCR signalling opens TRPV4 expressed in HEK293 cells</td>
<td>William Darby</td>
<td>438</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>439</td>
<td>The binding mode of SB269652: a novel bitopic ligand at the dopamine D2 receptor</td>
<td>Christopher Draper-Joyce</td>
<td>439</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>440</td>
<td>Antitumour actions of the synthetic Omega-3 17,18-epoxyeicosanoid acid in breast cancer cells</td>
<td>Herryiawan Ryadi</td>
<td>440</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>441</td>
<td>Glucocorticoids inhibit breast tumour cell migration but increase metastasis to the lung in a mouse model of breast cancer</td>
<td>Ebony Fietz</td>
<td>178</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>442</td>
<td>Identification of novel allosteric modulators of the α1A and α1B adrenoceptors</td>
<td>Angela Finch</td>
<td>442</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>443</td>
<td>Inhibition of human haematological malignant cell line growth by capsacin is not TRPV1-mediated</td>
<td>Dom Geraghty</td>
<td>443</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>444</td>
<td>Investigation of activation mechanism of TRPA1 ion channel</td>
<td>Lixiong Gu</td>
<td>444</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>445</td>
<td>A methanolic extract of propolis collected from the Australian native stingless bee, Tetragona carbonaria, scavenges free radicals and inhibits 5-lipoxygenase activity in vitro</td>
<td>Karina Hamilton</td>
<td>445</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>446</td>
<td>Opioid inhibition of cAMP in an optimised cell based assay</td>
<td>Dilanthi Herath</td>
<td>446</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>447</td>
<td>Activity of a novel alpha-conotoxin Ls1A, isolated from Conus limus</td>
<td>Marco Inserria</td>
<td>447</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>448</td>
<td>Assessment of two-pore channels in MDA-MB-231 breast cancer cells</td>
<td>Asiyah H Jahidin</td>
<td>448</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>449</td>
<td>TRPV4 channels in basal-like breast cancer cells</td>
<td>Sri Y N Jamaludin</td>
<td>449</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>Board number</td>
<td>Abstract title</td>
<td>Presenter</td>
<td>Paper reference</td>
<td>Session theme</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------</td>
<td>-----------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>450</td>
<td>Characterisation of the hybrid orthosteric/allosteric bitopic nature of TBPP at the M1 muscarinic ACh receptor</td>
<td>Peter Keov</td>
<td>222</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>451</td>
<td>A novel analytical approach reveals a distinct pattern of stimulus bias for an antipsychotic drug at the dopamine D2 receptor</td>
<td>Carmen Klein Herenbrink</td>
<td>451</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>452</td>
<td>Development of a real-time, fluorescence based assay of mu-opioid receptor mediated inhibition of adenylate cyclase activity in Chinese hamster ovary cells</td>
<td>Alisa Knappen</td>
<td>452</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>453</td>
<td>In vitro assessment of ligand-mediated μ-opioid receptor interaction with Gproteins and β-arrestin 2</td>
<td>Ar-Leen Lam</td>
<td>453</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>454</td>
<td>The role of the secretory pathway calcium ATPases (SPCAs) in breast cancer</td>
<td>Jane Lee</td>
<td>176</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>455</td>
<td>Taking advantage of kinetic data: an alternative approach to obtain affinity estimates from GPCR-mediated intracellular calcium mobilization</td>
<td>Lauren May</td>
<td>455</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>456</td>
<td>The efficacy of kappa-opioid receptor agonists in pain and inflammation</td>
<td>Michael Morgan</td>
<td>456</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>457</td>
<td>Anti-inflammatory kinase inhibitors attenuate invasiveness and chemoresistance of glioblastoma cells</td>
<td>Lenka Muroz</td>
<td>457</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>458</td>
<td>Structure-function analysis of orthosteric, allosteric and bitopic ligand binding at adenosine A1 receptors</td>
<td>Anh Thi Ngoc Nguyen</td>
<td>177</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>459</td>
<td>Assessing the cytotoxicity of natural products on squamous cell carcinoma and human keratinocyte cell lines</td>
<td>Thao Nguyen</td>
<td>459</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>460</td>
<td>Alteration of SKBR3 cancer cell proliferation by silencing specific calcium pumps, channels and channel modulators</td>
<td>Elena Pera</td>
<td>460</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>461</td>
<td>Immunohistochemical analysis of PMCA2 expression in normal and malignant human breast tissues</td>
<td>Arneta Peters</td>
<td>461</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>462</td>
<td>Prediction of the GAG-binding interactions of four-helical cytokines</td>
<td>Maryam Sherkat Masoum</td>
<td>462</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>463</td>
<td>Calcium dependent regulation of multidrug resistance-associated protein 3 (MRP3/ABCC3) gene expression in a model of epithelial-mesenchymal transition (EMT)</td>
<td>Teneale Stewart</td>
<td>220</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>464</td>
<td>Investigating the mechanism of RXFP1 activation</td>
<td>Brad Hoare</td>
<td>464</td>
<td>Drug Discovery</td>
</tr>
<tr>
<td>503</td>
<td>Kavalactones: Novel positive modulators of α1/2/3/2L GABAA receptors</td>
<td>Emile Christensen</td>
<td>503</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>504</td>
<td>Rat model of varicella zoster virus (VZV) induced neuropathic pain</td>
<td>Vaskar Das</td>
<td>504</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>505</td>
<td>Balb/c, C57BL/6J and CBA mice: characterisation of a new population of MDMA (ecstasy) users</td>
<td>Jake Gordon</td>
<td>505</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>507</td>
<td>Attenuation of Toll-like receptor 4 reduces reward-like behaviours in mice</td>
<td>Jonathan Henry Jacobsen</td>
<td>170</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>508</td>
<td>Medication overuse headache is a manifestation of opioid induced hyperalgesia: A neuroimmune hypothesis and novel approach to treatment</td>
<td>Jacinta Johnson</td>
<td>508</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>509</td>
<td>Cannabinoid receptor interacting protein (CRIP1a) modulates CB1 receptor mediated GIRK channel activation in AIT-20 cells</td>
<td>Nikushi Karunarathne</td>
<td>509</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>510</td>
<td>Mechanical hyperalgesia, but not allodynia, is sustained through δ9-nicotinic ACh-receptor activity</td>
<td>Sarasa Mohammadi</td>
<td>510</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>511</td>
<td>Omega-conotoxins CVID and CVIE and two analogues display age-sensitive differences in biophysical properties in sensory neurons</td>
<td>Sveltia Murali</td>
<td>511</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>512</td>
<td>Analgesic efficacy and mode of action of a small molecule angiotensin type 2 receptor antagonist in a rat model of prostate cancer induced bone pain</td>
<td>Arjun Muridalaharan</td>
<td>169</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>513</td>
<td>TLR2/4KO mice do not display classical opioid receptor binding</td>
<td>Jacob Thomas</td>
<td>513</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>514</td>
<td>Ciguatoxin-induced cold allodynia is an acquired peripheral channelopathy involving preferential activation of TRPA1-expressing nociceptors</td>
<td>Irina Vetter</td>
<td>514</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>515</td>
<td>The influence of surgery-induced inflammation and isoflurane anaesthesia on postoperative cognitive outcome</td>
<td>Catherine Wood</td>
<td>515</td>
<td>Neuro- and Behavioural Pharmacology</td>
</tr>
<tr>
<td>516</td>
<td>Combinational therapy with oxycodone and zolendronic acid in inflammatory arthritis: The role of cytokines in acute and chronic inflammation</td>
<td>Waltraud Binder</td>
<td>516</td>
<td>Inflammation/Respiratory Medicine</td>
</tr>
<tr>
<td>517</td>
<td>Small airway reactivity to constrictors is differentially altered by chronic and acute inflammatory stimuli</td>
<td>Chantal Donovan</td>
<td>221</td>
<td>Inflammation/Respiratory Medicine</td>
</tr>
<tr>
<td>518</td>
<td>Choice of contractile agonist influences dilator efficacy in small airways in mouse lung slices</td>
<td>Meaghan FitzPatrick</td>
<td>518</td>
<td>Inflammation/Respiratory Medicine</td>
</tr>
<tr>
<td>519</td>
<td>Effect of anti-oxidants on influenza A infection in cigarette smoke-exposed mice</td>
<td>Prasanthi Gunasinghe</td>
<td>519</td>
<td>Inflammation/Respiratory Medicine</td>
</tr>
<tr>
<td>520</td>
<td>Investigating the role of PTEN in airway epithelial inflammation and remodelling in Chronic Obstructive Pulmonary Disease (COPD)</td>
<td>Amanda Vannitamby</td>
<td>520</td>
<td>Inflammation/Respiratory Medicine</td>
</tr>
<tr>
<td>521</td>
<td>Parents’ perspectives about factors influencing adherence to pharmacotherapy for attention-deficit hyperactivity disorder (ADHD)</td>
<td>Rana Ahmed</td>
<td>521</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>522</td>
<td>Utilisation of antihypertensive medication in elderly hospitalized patients</td>
<td>Tanig Athawassi</td>
<td>522</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>523</td>
<td>Using academic detailing to support nurses’ knowledge and confidence around antipsychotic drugs in dementia</td>
<td>Beate Kristine Antonsen</td>
<td>523</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>524</td>
<td>Interprofessional learning: impact on collaboration and attitudes towards health care teams</td>
<td>Sinthia Bosnic-Anticevich</td>
<td>524</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>525</td>
<td>Perceived barriers to pharmaceutical services by clients with mobility, vision and hearing disabilities</td>
<td>Victor Chuang</td>
<td>525</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>526</td>
<td>ASCIA Anaphylaxis Training for Pharmacists</td>
<td>Rhonda Clifford</td>
<td>526</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>527</td>
<td>Risk factors for chlamydia: A survey of pharmacy-based emergency contraception consumers in Australia</td>
<td>Rhonda Clifford</td>
<td>527</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>528</td>
<td>Off-label and unlicensed prescribing in a Western Australian paediatric population</td>
<td>Petra Czarniak</td>
<td>528</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>529</td>
<td>Medines and their management among the older-aged living independently in leasehold retirement villages</td>
<td>Sheila Doggrel</td>
<td>529</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>530</td>
<td>The role of a medication incident reporting system in monitoring and signalling medication safety risks in primary care</td>
<td>Khaled Eddie</td>
<td>530</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>531</td>
<td>The perceived efficacy of non-prescription medications used by women who experience PMS</td>
<td>Kwang Choon Yee</td>
<td>531</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>Board number</td>
<td>Abstract title</td>
<td>Presenter</td>
<td>Paper reference</td>
<td>Session theme</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-----------</td>
<td>-----------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>561</td>
<td>Identification of Medication Errors amongst healthcare providers and academics in Denpasar Bali</td>
<td>Desak Ernawati</td>
<td>561</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>562</td>
<td>Assessment of chronic pain in the community</td>
<td>Joy Spark</td>
<td>562</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>563</td>
<td>A taste of your own medicine: prevalence of symptoms and self-medication in the community</td>
<td>James Green</td>
<td>563</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>564</td>
<td>The effect of weak English skills on academic performance, and the effectiveness of English language screening and remedial help for pharmacy students</td>
<td>James Green</td>
<td>564</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>565</td>
<td>The impact of the rescheduling of combination analgesics containing codeine on the practice of pharmacists</td>
<td>Joy Spark</td>
<td>565</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>566</td>
<td>Commercial influences on community pharmacist recommendations - impact of the Extended and Accelerated Price Disclosure</td>
<td>Lillian Huang</td>
<td>566</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>567</td>
<td>Concomitant use of alcohol and sedative-hypnotics in middle and older aged people: a systematic review</td>
<td>Simon Bell</td>
<td>567</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>568</td>
<td>The Medwise Model - is it working in the Bay of Plenty?</td>
<td>Maree Jensen</td>
<td>568</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>569</td>
<td>Timing of the drug administration in clinical practice in Australia</td>
<td>Gagandeep Kaur</td>
<td>569</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>570</td>
<td>Current tapering recommendations for discontinuing psychotropic medications: a systematic review</td>
<td>Greg Kyle</td>
<td>570</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>571</td>
<td>Creating a smoke free University of Canberra</td>
<td>Greg Kyle</td>
<td>571</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>572</td>
<td>Development of health professional relationships in an Interprofessional Learning workshop</td>
<td>Sofia Manitsakis</td>
<td>572</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>573</td>
<td>“I just have to get off my arse” Barriers to medication adherence in young adults with chronic disease</td>
<td>Cobie McQueen</td>
<td>573</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>574</td>
<td>Community pharmacy-consumer/carer relationships are key in mental health care</td>
<td>Amary Mey</td>
<td>574</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>575</td>
<td>Utilization of actigraphy to assess undiagnosed sleep disturbances among healthy adults in home-based settings</td>
<td>Zaswiza Mohamad Noor</td>
<td>575</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>576</td>
<td>Investigating pharmacists interventions on discharge from hospital</td>
<td>Rayan Nahas</td>
<td>576</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>577</td>
<td>Developing a methodology to target and individualise interventions to improve medication adherence in community pharmacies</td>
<td>Thi-My-Uyen Nguyen</td>
<td>577</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>578</td>
<td>What are validated adherence scales really measuring?: A systematic review</td>
<td>Thi-My-Uyen Nguyen</td>
<td>578</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>579</td>
<td>Community pharmacists’ awareness of secondary prevention of cardiovascular disease: a preliminary study</td>
<td>Harini Pushpattasi</td>
<td>579</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>580</td>
<td>Career perspectives of final year Australian pharmacy students</td>
<td>Grace Shen</td>
<td>580</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>581</td>
<td>The use of herbal medicines in lactation among breastfeeding women in Western Australia: A population-based survey</td>
<td>Tin Fei Sim</td>
<td>581</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>582</td>
<td>Accessibility of compounded progesterone products</td>
<td>Joy Spark</td>
<td>582</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>583</td>
<td>Medicines prices to patients in Australia</td>
<td>Loc Thai</td>
<td>583</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>584</td>
<td>Medicine information accompanying OTC medicines: Do labels and leaflets adequately support safe and appropriate use?</td>
<td>Vivien Tong</td>
<td>584</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>585</td>
<td>Usage of heart failure medications in frail and robust older inpatients</td>
<td>Kristina Waddell</td>
<td>585</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>586</td>
<td>Retrospective review of initial management of febrile neutropenia at Flinders Medical Centre: time to first dose of antibiotic therapy and risk stratification</td>
<td>Diane Watson</td>
<td>586</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>587</td>
<td>A longitudinal study of constipation and laxative use in a community-dwelling elderly population</td>
<td>Barry Wther</td>
<td>587</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>588</td>
<td>The impact of a pharmacist-led educational program on the psychotropic medication knowledge of aged care nurses</td>
<td>Juanita Westbury</td>
<td>588</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>589</td>
<td>Investigation of benzodiazepine utilisation in older people admitted to secondary care</td>
<td>Jackson Crown</td>
<td>589</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>590</td>
<td>An audit of antibiotic prescribing for urinary tract infections in palliative care hospices in Scotland</td>
<td>Barbara Wimmer</td>
<td>590</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>591</td>
<td>A systematic review of healthcare interventions for asthma management during pregnancy</td>
<td>Elda Zarrina</td>
<td>591</td>
<td>Pharmacy Practice</td>
</tr>
<tr>
<td>615</td>
<td>Genetic polymorphisms of the CNS immune and opioid signalling pathways are associated with morphine requirements after caesarean delivery</td>
<td>Daniel Barnett</td>
<td>615</td>
<td>Pharmacogenomics</td>
</tr>
<tr>
<td>616</td>
<td>The pharmacogenomics knowledge, education, practice and attitudes of hospital pharmacists in Adelaide, South Australia</td>
<td>Mafakda Dias</td>
<td>616</td>
<td>Pharmacogenomics</td>
</tr>
<tr>
<td>617</td>
<td>A pharmacogenomic study investigating outcomes in advanced non-small cell lung cancer patients receiving paclitaxel and carboplatin therapy with a focus on ethnic differences</td>
<td>Benjamin Harris</td>
<td>617</td>
<td>Pharmacogenomics</td>
</tr>
<tr>
<td>618</td>
<td>Impact of recipient and donor multidrug resistance protein 2 genetic variability on mycophenolic acid pharmacokinetics following kidney transplantation</td>
<td>Zaipul Md Dom</td>
<td>618</td>
<td>Pharmacogenomics</td>
</tr>
<tr>
<td>632</td>
<td>Changes in vimentin distribution accompany acrolein toxicity in epithelial lung cells: Association with protein adduct distribution</td>
<td>Kimberley Burton</td>
<td>632</td>
<td>Toxicology</td>
</tr>
<tr>
<td>633</td>
<td>Acute acrolein exposure produces molecular, morphologic and functional changes in airway epithelium as investigated in a novel mouse perfused tracheal system</td>
<td>Esther Cheah</td>
<td>633</td>
<td>Toxicology</td>
</tr>
<tr>
<td>634</td>
<td>Expression of TRRs and SgAPF in the rat intestine following chemotherapy for cancer and relationship to put toxicity and central pain behaviour</td>
<td>Janet Coller</td>
<td>634</td>
<td>Toxicology</td>
</tr>
<tr>
<td>635</td>
<td>Safety and toxicity profile of fenugreek</td>
<td>Song Huan Eow</td>
<td>635</td>
<td>Toxicology</td>
</tr>
<tr>
<td>636</td>
<td>Structure toxicity studies of drugs implicated in immune mediated idiosyncratic hepatotoxicity</td>
<td>Samuel Ho</td>
<td>636</td>
<td>Toxicology</td>
</tr>
<tr>
<td>637</td>
<td>Mulga snake (Pseudochis australis) envenoming: a spectrum of myotoxicity, anticoagulant coagulopathy, haemolysis and the role of early antivenom therapy -Australian Snakebite Project (ASP-18)</td>
<td>Christopher Johnston</td>
<td>637</td>
<td>Toxicology</td>
</tr>
<tr>
<td>638</td>
<td>Resveratrol does not protect against paracetamol-induced cell death in mouse primary hepatocytes</td>
<td>Alice Kane</td>
<td>638</td>
<td>Toxicology</td>
</tr>
<tr>
<td>639</td>
<td>The effect of ageing on paracetamol pharmacokinetics and toxicity</td>
<td>John Mach</td>
<td>639</td>
<td>Toxicology</td>
</tr>
<tr>
<td>640</td>
<td>Isolation and characterisation of a procoagulant serine proteinase from the venom of the Eyelash pit viper, Bothriechis schlegelii</td>
<td>Emily Maguire</td>
<td>640</td>
<td>Toxicology</td>
</tr>
<tr>
<td>641</td>
<td>The teratogenic effect of deltofliide during rat limb development and association with drug-induced bradycardia and hypoxia</td>
<td>Diana Oakes</td>
<td>641</td>
<td>Toxicology</td>
</tr>
<tr>
<td>642</td>
<td>Chronic low dose exposure to STX inhibits neurite outgrowth</td>
<td>Kate O’Neill</td>
<td>642</td>
<td>Toxicology</td>
</tr>
<tr>
<td>643</td>
<td>Intravenous Lipid Emulsion does not improve haemodynamics or survival and increases drug concentrations in a rodent model of oral amitriptiline poisoning</td>
<td>Danielle Perichon</td>
<td>643</td>
<td>Toxicology</td>
</tr>
<tr>
<td>644</td>
<td>In vitro assessment of chemical sensitisation potential using the human cellline activation test (h-CLAT)</td>
<td>Chiu Lin Wong</td>
<td>644</td>
<td>Toxicology</td>
</tr>
</tbody>
</table>
Proton Pump Inhibitors - Robust Evidence of Public Health Hazard, or Just Plain Confounding?
Yoon K Loke, Senior Lecturer in Clinical Pharmacology, Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, United Kingdom.

Proton pump inhibitors (PPIs) are very widely used in healthcare settings for prevention or treatment of upper gastrointestinal disease. The perceived safety of PPIs may have led prescribers and patients to accept extended durations and indications for such therapy without any major concerns. However, PPIs have been dogged with controversy recently, with a number of drug safety updates from the regulatory authorities that warn of potentially serious harm. These problems include reported associations between PPI use and increased risks of fracture, as well as Clostridium difficile infection (CDI). A potential drug interaction between clopidogrel and PPIs has also been hotly debated, with suggestions that use of certain PPIs can significantly elevate the risk of adverse cardiovascular events. However, proponents of PPIs argue the gastrointestinal benefits of PPIs are substantial and that the signals for harm stem from studies that are methodologically weak.

This presentation will discuss evidence from several recent systematic reviews of adverse effects, and provide an update on what is known (and not known) about the benefits and harms of PPIs.

Key References:

Transporter-based drug-drug interactions – principles and examples
Jashvant (Jash) D. Unadkat1, Department of Pharmaceutics, School of Pharmacy, University of Washington1, Seattle, USA

Transporter-based drug interactions (DDI) can be complex and difficult to predict due to their tissue, cell, and cell-membrane specific localization. Transporter-based DDI will be clinically relevant for victim drugs with relatively narrow therapeutic window and where transporters are the rate-limiting step in their disposition (including tissue distribution). In my presentation, I will illustrate the concepts of rate-limiting step and “cloaked” DDI using data from animal and human studies. Supported by NIH (multiple grants), UWRAPT, Pfizer Inc.
Role of transporters in mediating hepatobiliary clearance of drugs

Kim LR Brouwer, Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Hepatic transport proteins facilitate the uptake and excretion of many drugs, and are well-recognized sources of potential drug-drug interactions (DDIs). Inhibition of hepatic uptake and/or efflux transporters may alter drug exposure in the liver and/or the systemic circulation leading to changes in efficacy and/or toxicity. A technique coupling gamma scintigraphy with a customized oroenteric catheter and specialized clinical protocol was developed to accurately quantify hepatic uptake, exposure, and biliary clearance (Cl\textsubscript{biliary}) of drugs in humans [Ghibellini et al (2007) Clin Pharmacol Ther 81:406-413]. Using this approach, the effect of ritonavir, a potent inhibitor of hepatic transport proteins, on the hepatobiliary disposition of 99mTc-mebrofenin was investigated in healthy volunteers. The efficient hepatic uptake of 99mTc-mebrofenin is mediated by OATP1B1 and OATP1B3; 99mTc-mebrofenin is rapidly excreted into bile unchanged by MRP2 [Ghibellini et al (2008) Pharm Res 25:1851-1860]. Ritonavir (multiple-dose: 2x300mg) significantly increased systemic 99mTc-mebrofenin exposure compared to control (4464±1861 vs. 1970±311 nCi*min/mL; mean±s.d.) without affecting overall hepatic exposure or biliary recovery. A semi-physiologically-based pharmacokinetic model was developed based on the blood, liver and bile data to elucidate potential sites/mechanisms of the 99mTc-mebrofenin-DDI, and in vitro studies were conducted. A novel extrahepatic distribution compartment was required to characterize 99mTc-mebrofenin disposition in humans. Ritonavir inhibited 99mTc-mebrofenin accumulation in human sandwich-cultured hepatocytes (IC\textsubscript{50}=3.46±1.53 \mu M). Despite ritonavir accumulation in hepatocytes, intracellular binding was extensive (97.4%), which limited interactions with MRP2-mediated biliary excretion. In vitro data supported conclusions from modeling/simulation that ritonavir inhibited 99mTc-mebrofenin hepatic uptake, but not biliary excretion, at clinically relevant concentrations. This integrated approach utilizing clinical and in vitro data, and modeling/simulation, emphasizes the importance of hepatic and extrahepatic drug distribution, assessment of inhibitory potential in relevant in vivo systems, and intracellular unbound concentrations to accurately predict the functional consequences of potential hepatic transport DDIs. This research was supported by NIH GM41935.

Role of organic cation transporters in mediating oral drug absorption

Dhiren R Thakker, William R Proctor, Tianxiang Han, Chester Costales, David Bordet, ShinHong Kang, Ravindra Varma Alluri, and Ruth Everett, Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, The University of North Carolina, Chapel Hill, USA

Intestinal epithelium is a formidable barrier to oral drug absorption. The tightly packed columnar cells with narrow intercellular (paracellular) space further restricted by the presence of tight junctions, present a physical barrier to hydrophilic compounds, particularly those with net charge over the pH range within the intestinal lumen. This is because the hydrophilic and/or charged compounds cannot cross the lipid bilayer of cell membranes of the enterocytes, and cannot traverse the narrow paracellular space, restricted by the presence of tight junctions. Therefore, transporters play an important role in the oral absorption of hydrophilic charged compounds. Specifically, we have asked the question why certain hydrophilic cationic drugs have high bioavailability despite their hydrophobicity and net positive charge. Hence, we have investigated the mechanism of absorptive transport of the hydrophilic weak bases H2-receptor antagonists, ranitidine and famotidine, as well as the leading anti-diabetic drug, metformin, which is permanently charged at all physiologic pHs, in various cellular, tissue, and in vivo models. Our studies showed that organic cation transporters (OCTs), specifically OCT1, play an important role in oral absorption of these drugs. However, paracellular transport also contributes significantly to their intestinal absorption. Thus, our studies in the Caco-2 cell monolayers showed that almost 50% of ranitidine absorptive transport was transepithelial and was mediated by cation-selective transporters, whereas remaining 50% of absorptive transport was paracellular. In contrast, almost 90% of metformin crosses Caco-2 cell monolayers in the absorptive direction via the paracellular route, although cation-selective transporters mediate apical uptake of the drug into the cells. A novel mechanism for intestinal absorption of metformin will be described that involves repeated cycling between enterocytes and intestinal lumen, mediated by multiple apical cation-selective transporters, and paracellular absorptive transport that is assisted by claudins in tight junctions of the intestinal epithelium.

Role of transporters in mediating hepatobiliary clearance of drugs

Kim LR Brouwer, Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Hepatic transport proteins facilitate the uptake and excretion of many drugs, and are well-recognized sources of potential drug-drug interactions (DDIs). Inhibition of hepatic uptake and/or efflux transporters may alter drug exposure in the liver and/or the systemic circulation leading to changes in efficacy and/or toxicity. A technique coupling gamma scintigraphy with a customized oroenteric catheter and specialized clinical protocol was developed to accurately quantify hepatic uptake, exposure, and biliary clearance (Cl\textsubscript{biliary}) of drugs in humans [Ghibellini et al (2007) Clin Pharmacol Ther 81:406-413]. Using this approach, the effect of ritonavir, a potent inhibitor of hepatic transport proteins, on the hepatobiliary disposition of 99mTc-mebrofenin was investigated in healthy volunteers. The efficient hepatic uptake of 99mTc-mebrofenin is mediated by OATP1B1 and OATP1B3; 99mTc-mebrofenin is rapidly excreted into bile unchanged by MRP2 [Ghibellini et al (2008) Pharm Res 25:1851-1860]. Ritonavir (multiple-dose: 2x300mg) significantly increased systemic 99mTc-mebrofenin exposure compared to control (4464±1861 vs. 1970±311 nCi*min/mL; mean±s.d.) without affecting overall hepatic exposure or biliary recovery. A semi-physiologically-based pharmacokinetic model was developed based on the blood, liver and bile data to elucidate potential sites/mechanisms of the 99mTc-mebrofenin-DDI, and in vitro studies were conducted. A novel extrahepatic distribution compartment was required to characterize 99mTc-mebrofenin disposition in humans. Ritonavir inhibited 99mTc-mebrofenin accumulation in human sandwich-cultured hepatocytes (IC\textsubscript{50}=3.46±1.53 \mu M). Despite ritonavir accumulation in hepatocytes, intracellular binding was extensive (97.4%), which limited interactions with MRP2-mediated biliary excretion. In vitro data supported conclusions from modeling/simulation that ritonavir inhibited 99mTc-mebrofenin hepatic uptake, but not biliary excretion, at clinically relevant concentrations. This integrated approach utilizing clinical and in vitro data, and modeling/simulation, emphasizes the importance of hepatic and extrahepatic drug distribution, assessment of inhibitory potential in relevant in vivo systems, and intracellular unbound concentrations to accurately predict the functional consequences of potential hepatic transport DDIs. This research was supported by NIH GM41935.
Inflammation resolution mediators in tumour growth and metastasis: good, bad and indifferent
Alastair G Stewart, Department of Pharmacology, University of Melbourne, Parkville, VIC

Inflammation, an ever-present feature of solid tumours, is a component of the tumour microenvironment that impacts on growth and metastasis. Angiogenesis, extracellular matrix composition/turnover, tumour cell migration and tumour immunity are influenced by the inflammatory milieu. Inflammation incites solutions that limit the duration and severity of the inflammatory response. Mediators of this inflammation resolution include annexin-1 and its N-terminal peptide, and a series of trihydroxy polyunsaturated fatty acid derivatives (lipoxins, protectins, resolvins and maresins). In certain tumour types infiltration with macrophages is associated with a negative prognosis. This association might reasonably be explained by an overall negative influence of the inflammation associated with the function of these tumour-associated macrophages (TAM). Tumours have been described as “wounds that do not heal”. Thus, inflammation-resolving, as well as pro-inflammatory mediators may accompany the persistent granulation tissue. Is the causal link between TAM and poor outcome explained by persistence of pro-inflammation or pro-resolution mediators? The evidence to address this issue is scant and a consistent picture has yet to emerge. Inflammation resolving mediators act directly on breast tumour epithelium to promote growth and metastatic processes (Yao et al 2008; Rondepierre et al, 2009; Khau et al, 2011). The importance of such direct influences as opposed to impacts on inflammation intensity/duration need to be defined in order to understand the potential of receptors for inflammation resolving mediators such as formyl peptide receptors (FPR), as targets for adjuvant therapy in treatment regimens for different solid tumours. Evaluation of the targeting potential of the FPR2 receptor subtype is further complicated by the diverse signaling evoked by pro-resolution ligands, annexin-1 and lipoxin A₄, compared to pro-inflammatory ligands, such as serum amyloid A.

Physiological correlates of biased receptor signaling: relevance to opioid drug action in health and disease
Laura M. Bohn, Kirsten M. Raehal, Chad E. Groer, John Streicher, Nicolette Ross, and Cullen L. Schmid.
Departments of Molecular Therapeutics and Neuroscience, The Scripps Research Institute, Jupiter, Florida, USA

Opioid analgesics, such as morphine, mediate their therapeutics actions by activating the mu opioid receptor (MOR) which is a G protein-coupled receptor (GPCR). Unfortunately, opioids also mediate most of their side-effects, such as respiratory suppression, constipation and dependence, by actions at this receptor. Like most GPCRs, the MOR interacts with beta-arrestins. Beta-arrestins serve as scaffolding proteins which can, upon agonist stimulation, lead to receptor desensitization of G protein coupling. Interestingly, beta-arrestins can also mediate GPCR signaling to alternate pathways thereby introducing a point at which GPCRs can be directed to signal based on their recruitment of the G protein. The degree of interaction between the GPCR and the beta-arrestin can also be influenced by the chemical composition of the ligand. Using beta-arrestin-2 KO mice, our laboratory has studied this protein’s contributions to MOR-mediated biological responses. We have found that in the absence of beta-arrestin2, morphine analgesia is enhanced and tolerance is attenuated suggesting that beta-arrestin2 plays a role in desensitizing signal transduction leading to antinociception. Other morphine-mediated behavioral responses, including dependence (antagonist-induced withdrawal), respiratory suppression and constipation are attenuated in this animal model suggesting that Barrestin2 may play a facilitatory role in the signaling underlying these responses. In this presentation we will present data examining additional signaling roles mediated by individual beta-arrestins such as those contributing to resensitization and ubiquitination, as well present some early developments in our drug discovery efforts to generate MOR agonists that are biased against beta-arrestin recruitment. According to extensive studies in the beta-arrestin2 mouse models, such a strategy may allow for the treatment of pain with fewer side-effects then seen with traditional opioid therapies. Funding for this work has been sponsored in part by the National Institute on Drug Abuse: R01DA14600, R01DA18860, R03DA025158 to LMB and F31DA021952.
Revealing the potential of GPCR bias signalling
Andrew B Tobin. Medical Research Council Toxicology Unit, Leicester, UK

One of the key features of GPCRs is the ability of each receptor to couple to numerous down-stream signalling pathways. This flexible signalling capacity underlies the fact that any one GPCR subtype might regulate very different physiological responses in different cell types. However, despite understanding the broad signalling capacity of GPCRs, recent thinking in this area has simplified GPCR signalling into essentially two pathways. The first is the traditional GPCR signalling via heterotrimeric G-proteins mediating phosphoinositide hydrolysis and calcium mobilisation. The second is signalling that lies down-stream of receptor phosphorylation and arrestin recruitment. Although simple, considering GPCRs in the context of bimodal signalling has helped with regard to investigating the concept of stimulus bias. This concept proposes that a GPCR agonist may stimulate a receptor so that both arms of the bimodal signalling pathway are equally activated. Such a ligand would have no stimulus bias. However, if a ligand induced a receptor conformation that allowed for one arm of the bimodal signalling pathway to be activated in preference to another then this ligand would show stimulus bias. The power of this concept is that if we were to understand the signalling modality employed by any particular GPCR to deliver a particular physiological, or indeed therapeutic, outcome then we would be able to rationally design GPCR ligands to bias the signalling towards that physiological/therapeutic outcome. In this way we would increase clinical efficacy and potentially reduce on-target, but undesired, side affects. How do we test if this promise can be delivered. In this presentation approaches to address this issue by using animal models together with molecular pharmacology approaches will be discussed.

Non-parametric population PK models and their use in individual patient care
Assoc Prof Michael Neely, University of Southern California, USA

Therapeutic drug monitoring in the 21st century can be much more sophisticated that simply comparing measured drug concentrations to a target range. In this session, attendees will be introduced to population modeling, and how it has advantages over traditional pharmacokinetic modeling that can improve patient care. We will discuss some of the differences between parametric and non-parametric population models, and show examples of how the latter are being used in practice to precisely control therapy in a flexible, powerful way. We will discuss some of the barriers to the use of such approach in routine care, as well as offer solutions to overcome many of those barriers.

Emerging trends in dose individualisation in clinical practice
Assoc Prof Jennifer Martin, The University of Queensland

Dose individualisation aims to achieve maximal efficiency with lowest toxicity and in some circumstances, lowest cost. It is an old science that is being revisited due to cost constraints, the availability of new pharmacogenetic data, new pharmacokinetic assays, new pharmacometric models and software.

Dose individualisation is undertaken by consideration of an individual patient's demographic, anthropomomeric and comorbidity profiles. Pharmacogenetic information for the particular drug may be available. This data enables a priori drug choice and dose individualisation. After initiating therapy, the drug response is evaluated using pharmacokinetic measurements (usually blood or urine concentrations), pharmacodynamic variables and/or clinical outcomes, and dose regimen altered. This data can be added to pharmacometric models to further refine and update dosing predictions.

This presentation will discuss the clinical use of these methodologies and their emerging use to improve dose individualisation in clinical practice. Reasons for success and failure of drug treatment and methodologies to improve successful dosing will be discussed.
Dose individualisation of dabigatran
Paul K L Chin1,2, Murray L Barclay1,2, Evan J Begg1,2. Department of Clinical Pharmacology, Christchurch Hospital1, NZ. Department of Medicine, University of Otago2, Christchurch, NZ.

Dabigatran is a novel oral anticoagulant that has been marketed as an advance on warfarin, with apparent advantages including the capacity for ‘fixed’ rather than ‘individualised’ dosing, few pharmacokinetic drug interactions, and no need for routine laboratory coagulation monitoring. However, we believe that the variance in its pharmacokinetics, in relation to its renal clearance and to the oral availability of its prodrug, dabigatran etexilate, necessitate a greater degree of dose individualisation than has been promoted. Currently, determination of appropriate dose-rates by prescribers is largely on the basis of estimating the patient’s glomerular filtration rate. However, it is unclear which glomerular filtration rate equation is best for guiding dabigatran etexilate dosing. Further, it is unclear how dosing should accommodate pharmacokinetic drug interactions, which is important as we have demonstrated that ~50% of patients discharged on dabigatran etexilate from our tertiary hospital institution have co-prescribed drugs that alter the oral availability of dabigatran etexilate. We propose that laboratory coagulation monitoring is a solution to these issues, and suggest targets for study and implementation.

The patient journey towards the end of life - patient and family accounts of ‘patient safety’
Aileen Collier¹, Rick Iedema¹, Centre for Health Communication¹, University of Technology, Sydney, NSW

Introduction. There is a dearth of research that is concerned specifically with end of life care and patient safety. Quality is driven by ‘patient safety’ mechanisms that prioritise the technical aspects of safety highlighting concepts such as ‘incident reporting’ and ‘medical error’ and the voices of dying patients and their families often go unheard. Aim. This study explores patient safety towards the end of life and the potential of visual methods to facilitate mutual understanding between patients, families and clinicians to address patient safety. This paper will present video narratives of what patient safety means for dying patients and their families from their own perspectives.

Methods. Patient participants were recruited via referral from specialist clinicians in a metropolitan hospital. Drawing from a framework of indigenous research ethics, collaborative video methodologies provided a medium to sensitively explore the complexities of end of life care. Methods were emergent and incorporated patients, families and clinicians in reflexively responding to their filmed narratives. In keeping with the underlying philosophy of the research opportunity will be provided within this presentation for the conference audience to engage reflexively with themed video excerpts. Firstly, this will demonstrate a key component of the study facilitating understanding of methodology. Second, connecting with the audience in this way will allow them to affectively engage with the research contributing to stakeholder knowledge and literacy of patient safety issues towards the end of life and informing the thesis.

Findings. Video offered an accessible and profound medium for collaborative sense making providing a reflexive space of engagement for patients, families and those caring for them. This research study found that the field of patient safety does not presently address the needs of dying people. Habitual care patterns expose dying patients and their families to pervasive harms along with those healthcare workers caring for them. Visual methods provide a disruptive innovation that challenges these normative and habitual rhythms of inattentiveness to healthcare safeties.

Towards a medicinewise Australia: the NPS approach to improving health literacy
Karen Kaye, Executive Manager, NPS MedicineWise, Sydney, NSW

Health literacy can be defined as the ability to access, understand, evaluate and communicate information as a way to promote, maintain and improve health in a variety of settings across the course of a person’s life.

To improve health literacy, it is necessary to engage effectively with both individuals and with systems.

The NPS approach involves:
• Finding ways to create awareness and promote curiosity amongst people in many different settings in order to start a conversation about the benefits and risks of medicines, medical tests and other medical options
• Finding ways to influence the influencers – opinion leaders and advocates in communities, the media and the health system
• Using messages and concepts that are simple and relevant to everyday life
• Working in partnership with expert intermediaries and others to promote and explain the importance of asking questions and seeking understanding about if, when and how to use medicines wisely
• Providing tools that help people put theory into action in a positive way
• Keeping the person and their carers at the centre and being wherever they are – in the community, in workplaces, in hospitals and other care settings, and at transitions of care in the health system.

Individuals seek and receive information about health choices from many sources, many of which are not evidence based. Health literacy develops iteratively over time and is enhanced when people are supported in relevant ways at times of particular information need. Navigating the health system and its myriad sources of information is a formidable task and health professionals have a critical role to play in helping people connect with reliable information and make good decisions wherever they are in their health journey. NPS information and tools can assist.
**The effect of ageing on paracetamol pharmacokinetics and toxicity in Fischer 344 rats**

John Mach\(^1\), Aniko Huizer-Pajkos\(^1\), Himesha Vandebona\(^3\), Victoria Cogger\(^3\), Catriona McKenzie\(^5\), David Le Couteur\(^3\), Brett Jones\(^1,2,3,6\), Sarah Hilmer\(^1,2\). Kolling Institute of Medical Research\(^1\), Sydney, Sydney, NSW; Royal North Shore Hosp\(^2\), Sydney, NSW; Sydney Medical School, Univ of Sydney, Sydney\(^3\), NSW; ANZAC Research Institute, Concord Hosp\(^2\), Sydney, NSW; Pathology Dept, Royal Prince Alfred Hosp\(^3\), Sydney, NSW; Gastroenterology Dept, Royal North Shore Hosp\(^2\), Sydney, NSW.

**Introduction.** In paracetamol overdose, conjugation pathways that form non-toxic products are saturated, leading to the formation of a toxic metabolite N-acetyl-p-benzo-quinone imine(NAPQI) by Cytochrome P450 2E1(CYP2E1). With excessive NAPQI levels, NAD(P)H quinone oxidoreductase(NQO1) activity and glutathione stores are exhausted resulting in hepatotoxicity. The effect of ageing on the metabolism of paracetamol has not been well characterised.

**Aims.** To characterise the degree of hepatotoxicity, expression and activity of key proteins involved in hepatic metabolism of paracetamol after administration of toxic doses to young and old rats.

**Methods.** Young adult(6±1 months) and old(26±2 months) male Fisher 344 rats were injected ip with 800mg/kg paracetamol(young n=8, old n=5) or saline(young n=9, old n=9) four hours prior to euthanasia by ip injection of ketamine (75mg/kg)/xylazine(10mg/kg). Serum ALT and liver histology were assessed to indicate the degree of hepatotoxicity. Serum paracetamol, paracetamol glucuronide, paracetamol sulfate and creatinine were measured. CYP2E1 protein expression and activity, NQO1 activity, UGT1A6 mRNA expression and glutathione levels were measured in liver samples.

**Results.** Old paracetamol treated rats had significantly lower serum ALT and higher serum paracetamol and paracetamol glucuronide levels, compared to paracetamol treated young rats. Amongst saline treated, compared to young, old rats had significantly lower CYP2E1 activity(~2fold) and higher NQO1 activity(~4fold)(p<0.05), with the same trend observed in animals treated with paracetamol. Hepatic glutathione did not differ with age, and was lower in animals treated with paracetamol than those with saline. There was no change in expression of UDP glucuronosyltransferase 1A6 mRNA with age or treatment. Compared to other groups, paracetamol treated old rats had higher serum creatinine(p<0.05).

**Discussion.** Despite higher serum levels of paracetamol, older rats appear to have decreased susceptibility to paracetamol-induced hepatotoxicity, which may be due to the reduced formation and enhanced metabolism of NAPQI. Older rats may be more susceptible to paracetamol-induced nephrotoxicity.

---

**Nox2β: A novel splice variant of Nox2 that promotes reactive oxygen species (ROS) production in macrophages**

Stavros Selemidis\(^1\), Craig B. Harrison\(^1\), Elizabeth Guida\(^1\), Paul T. King\(^2\), Christopher G\(^1\), Sobey & Grant R. Drummond\(^1\). Dept of Pharmacology, Monash University\(^1\), Clayton, VIC; Dept of Medicine/Respiratory Medicine, Monash Medical Centre\(^2\), Clayton, VIC.

**Introduction:** Nox2 oxidase is one isofrom in a family of seven NADPH oxidases that generate reactive oxygen species (ROS) and thereby contribute to physiological and pathological processes including host defense, redox signaling, immune function and oxidative tissue damage.

**Aims:** To establish whether alternative mRNA splicing gives rise to functionally relevant splice variants of Nox2 that modulate ROS production.

**Methods:** Western blotting, RT-PCR, DNA sequencing, quantitative real-time PCR and L-012-enhanced chemiluminescence to measure ROS production, were performed on a variety of mouse tissues and primary macrophages, as well as the immortalized macrophage cell line (RAW264.7) and human alveolar macrophages.

**Results and Discussion:** Immunoscreening for the presence of truncated Nox2 proteins identified a 30-kDa protein in lung, and in peritoneal and alveolar macrophages from wild-type mice, and in atherosclerotic aortas from APOE-/- mice. RT-PCR analysis of mRNA from mouse macrophages, and from human alveolar macrophages, identified a truncated Nox2 transcript which, upon sequence analysis, was found to be a product of the ‘exon skipping’ mode of alternative splicing, lacking exons 4-10 of the Nox2 gene. The predicted protein is comparable in size to that identified by immunoscreening and contains two transmembrane helices and an extended cytosolic C-terminus with binding sites for NADPH and the Nox organiser protein p47phox. Importantly, selective siRNA-mediated knockdown of the transcript reduced expression of the 30-kDa protein in macrophages, and suppressed phorbol ester-stimulated ROS production by 50 %. Hence, this study provides the first evidence that Nox2 undergoes alternative mRNA splicing to yield a 30-kDa protein – herein termed Nox2β – that regulates NADPH oxidase activity and the oxidative burst in macrophages from mice and humans. The discovery of Nox2β paves the way for future examination of its role in physiological and pathological processes, in particular cardiovascular and lung diseases.
**Screening potential skin sensitizers using high throughput direct peptide activity assay**

Sussan Ghassabian¹, Seyed Mojtaba Moosavi¹, Bruce Wyse¹, CIPDD, Univ of Queensland¹, Brisbane, QLD.

Introduction: Skin sensitizers and their metabolites are generally electrophilic and reactive towards nucleophilic sites on proteins. Among all amino acids, “Lysine and Cysteine” are relatively strong nucleophiles involved in hapten-protein interactions.

Aim: To develop an in vitro high-throughput LC-MS/MS method for measuring the depletion of a heptapeptide containing cysteine and lysine after incubation with a compound with unknown potential to cause skin sensitization reactions.

Method: Lactic acid, isopropanol, salicylic acid (non-sensitizer), geraniol, ethyl acrylate, eugenol (weak sensitizer), isoeugenol, 5-amino-2-methyl phenol (moderate sensitizer), 1-chloro-2,4-dinitrobenzene, and 3-methyl catechol (strong sensitizer) were incubated with a heptapeptide containing cysteine and lysine (Ac-NKKCDLF) at the ratio of 1 in 10 (peptide/test item). Following incubation at 37 °C, pH=7 (50 mM phosphate buffer) for 24h, the concentration of the peptide were measured using an optimised LC/MS/MS method. The mobile phase comprised 10 mM ammonium acetate buffer, pH=9.5 (A), and acetonitrile (B) on a Phenomenex; Gemini C18 column. The detector (API 3200) settings and mobile phase gradient were optimised to generate the maximum response.

Results: The results shown in the figure (n=5) were significantly correlated (Spearman r = -0.69, p < 0.05) with published in vivo data (Natsch and Gfeller, 2008; Gerberrick et al, 2007) derived from the Local lymph node assay which is the gold standard method for assessment of skin sensitisation potential.

Discussion: This in vitro method has the potential to reduce animal use for assessment of skin sensitisation potential of novel compounds. Recent legislative changes by the European Union banning of animal use for testing of cosmetics and through enforcement of REACH regulations is driving the need for a cost-effective in vitro skin sensitization assay.


Expression and Localisation of Pannexin-1 Hemichannels in Human Colon in Health and Disease

Erica F Diezmos 1, Shaun L Sandow 2, Irit Markus 1, D. Shevy Perera 3, Denis W. King 3, Paul P Bertrand 2, Lu Liu 1. Depts of Pharmacology 1 and Physiology 2, School of Medical Sciences, University of New South Wales, Sydney, Australia; Sydney Colorectal Associates 3, St George Hospital, Sydney, Australia.

Introduction. Pannexin-1 (Panx1) proteins function as channels for ATP release when coupled to purinergic receptors and have roles in many cellular processes such as blood flow regulation and immune function (Locovei et al, 2006; Pelegrin et al, 2006). However, there are limited studies investigating their potential role in the human intestine.

Aims. The aim of the present study was to characterise Panx1 expression and distribution in the human colon and evaluate its potential involvement in inflammatory bowel diseases (IBD).

Methods. Human colon samples were dissected into mucosa and muscle components, and evaluated separately for Panx1 gene expression by real-time PCR and protein expression by Western blotting. Immunohistochemistry was conducted to localise the cellular distribution of Panx1 in intact tissues.

Results. In ulcerative colitis (UC) muscle, Panx1 mRNA expression showed a 3.5-fold reduction compared to control muscle (P=0.002, n=9-10), but no change was seen in UC mucosa. In contrast, a significant reduction of Panx1 mRNA was observed in both Crohn’s disease (CD) muscle and mucosa (2.7- and 1.8-fold reduction, respectively; P<0.05, n=6-11). There was a reduced Panx1 protein expression in CD muscle, but no change in CD mucosa, UC muscle or UC mucosa. In control, Panx1 immunoreactivity was localised to enteric ganglia, blood vessel endothelium, erythrocytes, epithelial cells and goblet cells. IBD samples showed a similar overall pattern of Panx1 staining, but significant Panx1 positive lymphocyte infiltrates were seen at the sites of inflammation. Furthermore, in UC myenteric ganglia, there was a significant reduction in Panx1 staining.

Discussion. The wide-spread presence of Panx1 in the colon suggests that they may play an important role in mediating gut function and changes to Panx1 distribution in disease suggest it may have a role in IBD pathophysiology.


Neurokinin A potentiates spontaneous and purinergic evoked smooth muscle contraction and bladder afferent activity responses via activation of mouse bladder urothelial and detrusor NK2 receptors.

Luke Grundy¹, Russ Chess-Williams¹. HSM, Bond University¹, Gold Coast, QLD.

Introduction. Neurokinin A (NKA) and its receptor, Tachykinin 2 (NK2), have been postulated to have an essential role in controlling normal bladder function. NK2 elicits contractions of human detrusor strips (Zeng et al, 1995) whilst it initiates a micturition reflex in Rats (Lecci et al, 1998). The nature of tachykinin induced bladder control, however, maintains poorly understood.

Aims. The aim of my study was to investigate the functional role of NKA and the NK2 receptor on mouse bladder function.

Methods. Mouse bladder sensory nerve and muscle activity were recorded using an in-vitro preparation which enables the simultaneous recordings of mouse afferent nerve firing and intravesical pressure. Bladders were continually perfused (30μl/min) with saline or NKA (10nM) and filled to a final pressure of 40mm/Hg every ten minutes. In separate experiments, the purinergic agonist αβMe-ATP (30μM) was administered to partially distended (12mmHg) bladders in the absence and presence of NKA (3nM). Data were compared using Student’s t-tests as appropriate.

Results. NKA elicits dose dependent detrusor contractions and an increase in afferent nerve activity which is completely blocked by the NK2 antagonist GR159897 (100nM) and Nifedipine (1μM). αβMe-ATP evoked a profound increase in afferent discharge and a concurrent contraction which returned to baseline over 2-3min. In the presence of NKA (3nM), αβMe-ATP contraction responses are significantly increased (peak contraction above baseline 22.56±3.19mmHg vs 16.34±2.62mmHg in controls, n=5, p<0.01) which was blocked by GR159897 (100nM). Spontaneous detrusor contractions and afferent nerve activity are dramatically potentiated by intravesical NKA (10nM).

Discussion. Actions of NKA within the bladder are limited to the urothelium and smooth muscle with no independent effects of NKA on afferent nerve activity. The results suggest there is a functional link between activation of urothelial NK2 receptors and detrusor contraction, potentially through the release of urothelial factors such as ATP.


Inflammatory biomarkers predict response and toxicity to FOLFOX in colorectal cancer patients.

Thien P Huynh¹, Sarah A Randall², Wei Chua³, Mark P Molloy², Stephen J Clarke⁴ and Kellie Charles¹.

Discipline of Pharmacology, Univ of Sydney¹, Camperdown, NSW; Australian Proteome Analysis Facility (APAF), Macquarie Univ², Macquarie Park, NSW; Dept of Medical Oncology, Liverpool Hospital³, Liverpool, NSW; Dept of Medical Oncology, Royal North Shore Hospital⁴, St Leonards, NSW.

Introduction. FOLFOX is the first-line chemotherapy regimen used to treat metastatic colorectal cancer patients. Good response to FOLFOX significantly can extend patient survival by 2.5 years. However, there is large and unpredictable variability in drug response and toxicity between patients.

Aims. To identify novel biomarkers that predict FOLFOX response and toxicity in metastatic colorectal cancer patients.

Methods. Plasma proteomics (iTRAQ and SRM-MS) was used to identify and quantitate proteins in plasma samples collected from patients prior to and after treatment with FOLFOX. Protein expression was compared to neutrophil-lymphocyte NLR status (high > 5, low <5) as a surrogate marker of survival. Statistical analysis was conducted using the ratio of geometric means and ANOVA to identify proteins that are significantly up/down-regulated.

Results. Inflammatory proteins from various key pathways involved in carcinogenesis (survival, acute phase response, complement and coagulation) were significantly upregulated in patients with high NLR (lower survival). Whilst several proteins involved in the insulin-like growth factor system were significantly upregulated after 2 days of chemotherapy in patients with high NLR. These results are summarised in the following table.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Fold change (high vs low NLR)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement factor H-related protein 2</td>
<td>3.15</td>
<td>0.003</td>
</tr>
<tr>
<td>Properdin</td>
<td>1.29</td>
<td>0.03</td>
</tr>
<tr>
<td>Protein S100-A9</td>
<td>3.56</td>
<td>0.02</td>
</tr>
<tr>
<td>Coagulation factor X</td>
<td>1.20</td>
<td>0.006</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein</th>
<th>Fold change (after vs before chemotherapy)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin-like growth factor binding protein 3</td>
<td>1.18</td>
<td>0.04</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 4</td>
<td>1.18</td>
<td>0.03</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 6</td>
<td>1.78</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Discussion. Inflammation is crucial in driving carcinogenesis. This study has identified proteins that may be useful as biomarkers for response and toxicity and has the potential to offer a personalised approach to patient selection for treatment.
What parents want to know about attention-deficit hyperactivity disorder (ADHD)- A qualitative investigation
Rana Ahmed1, Jacqueline Borst2, Yong Cheng Wei3, Parisa Aslani1. Faculty of Pharmacy, Univ of Sydney1, Sydney, NSW; Univ of Utrecht2, Utrecht, Netherlands; Univ of Nottingham3, Nottingham, United Kingdom.

Introduction. Attention-deficit hyperactivity disorder (ADHD) is the most pervasive paediatric psychological condition. Stimulant medicines are used as first-line treatment, but are surrounded by controversy, placing parents in a difficult position to make treatment decisions. Parents must have access to comprehensive, relevant information about ADHD and its treatments to empower them to make informed decisions on their child’s behalf.

Aims. To explore the ADHD-related information sources, knowledge and information needs of parents with children affected by ADHD.

Methods. Focus groups (n=3) were conducted with 16 parents recruited from metropolitan Sydney areas by a market research company. Each focus group lasted 1-1.5 hours and was audio-recorded, transcribed verbatim and thematically content analysed.

Results. Most parents sourced verbal information from health-care professionals (HCPs) focused on ADHD medicines rather than the condition itself. Other sources of information were the internet followed by school staff and, lastly, pharmacists. Generally, parents had limited ADHD-related knowledge prior to their child’s diagnosis and perceived ADHD medicines in a negative context. Parents reported improved knowledge after their child’s diagnosis, but expressed dissatisfaction with information accessed which was often technical and not tailored to their child’s needs. They requested increased availability of support groups and tools to assist them in sourcing information from HCPs during consultations, such as question prompt lists.

Discussion. Parents in this study appeared to have limited information about ADHD or its treatments, with current sources of information and support not meeting their needs. For ADHD medicines to be used safely and effectively, it is essential that future research focuses on providing parents with avenues to access relevant, reliable information and support in order to empower them to make the best decision for their child.

The influence of disease and other factors on adherence
Piyanan Assawasuwannakit1, Rhiannon Braund1 & Stephen B Duffull1. School of Pharmacy, Univ of Otago1, Dunedin, NZ.

Introduction. Poor adherence remains a common barrier to achieving optimal therapeutic outcomes. Common forms of poor adherence include delayed initiation, early discontinuation or non-persistence and various patterns of incorrect dosing including missed doses and drug holidays (Blaschke et al, 2012). Medication event monitoring system (MEMS) devices have been used to record exact times that a medication bottle is opened (Cramer et al., 1989). It has been reported that poor adherence appeared to share similarities across different disease states (Urquhart and De Klerk, 1998). However little is known about the independent influence of disease and other patient characteristics (factors) on adherence.

Aims. The aim of this project is to evaluate the independent influence of various factors, including disease, dosing regimen and patient characteristics, on adherence.

Methods. A literature search was conducted to retrieve adherence studies using MEMS devices. Studies were categorised into different therapeutic areas. Only the two most commonly studied therapeutic areas were selected. Data were extracted from each study and analysed using a model based meta-analysis technique. This technique provided an estimate of the combined and independent influence of each factor on adherence.

Results. The most commonly recorded adherence criterion was percentage of prescribed doses taken per day. The therapeutic areas chosen were HIV (23 studies) and hypertension (12 studies). The statistically significant factors were disease, age and dosing regimen. The independent influences of each factor on adherence were: an increase in adherence of approximately 8% per 10 year increase of age, a 25% reduction from once to thrice daily dosing, and that HIV patients were 6% more adherent than patients with hypertension.

Discussion. This analysis provides the first evidence for the independent effects of various factors. It was found that the influence of disease while statistically significant was not clinically significant, however, and interestingly adherence improved with age.

**Beyond expectations? Do pharmacists perform clinical interventions when carrying out adherence support medication reviews?**

Ernieda md Hatah1,2, June M Tordoff1, Stephen B Duffull1, Rhiannon Braund1. School of Pharmacy, University of Otago1; Dunedin, Faculty of Pharmacy, Universiti Kebangsaan Malaysia2, Kuala Lumpur, Malaysia. (introduced by Rhiannon Braund, University of Otago, Dunedin).

Introduction. In New Zealand, pharmacists are funded to provide adherence support via a service called Medication Use Review and Adherence Support (MUR). This is not intended to be a clinical review. However, improving adherence ignores a key role that pharmacists play in ensuring optimal prescribing and use of medications, patient safety, and improving patient health outcomes.

Aims. The aim of this study was to determine the types of interventions provided by pharmacists while conducting MURs and to identify those interventions that may be considered to be beyond adherence support.

Methods. A single district health board that funds MUR services was identified. Those pharmacies that provided MURs during the funded period (from 2007 – current) were invited to participate. All consultations that had been documented were scanned on site and the data extracted was categorised according to the Pharmaceutical Care Network Europe (PCNE) Classification Scheme for Drug Related Problems v 6.2.

Results. Consultation records for 353 individual patients were obtained. Of these patients, 56.4% were female and the median age was 73 years. A total of 886 drug related problems were identified and resulted in a total of 844 interventions. The most common intervention was “patient counselling” (20%) followed by “compliance packaging provided” (16%), however the third most common intervention was directed at the prescriber level “recommendation to change medication” (11%).

Discussion. In this study pharmacists were found to be performing beyond the expected and funded level of the MUR service, by providing clinical interventions. While the current specifications surrounding MUR services are patient focused, they are based on the assumption of “optimal” prescribing. The current service specification does not support pharmacists in their role of identifying medication appropriateness, effectiveness and promoting patient safety. (282)

Asthma management in intellectual disability (ID) – identifying opportunities for the pharmacist
Sharon R Davis,1 Seeta Durvasula,1 Diana Merhi,2 Daniela Traini,1 Paul M Young,1 Sinthia Z Bosnic-Anticevich1. Faculty of Pharmacy, The University of Sydney1, Sydney, NSW; Centre for Disability Studies, The University of Sydney1, Sydney, NSW; Synergy Medical Practice2, St Leonards, NSW; Woolcock Institute of Medical Research, The University of Sydney1, Sydney, NSW.

Introduction. The inhalational route is the foundation of asthma treatment, however inhaler technique is complex, and optimal technique rarely achieved. Australian data indicate 15% of persons with ID have asthma. Due to physical and/or cognitive deficits, persons with ID may present a management challenge regarding inhaler use, however no evidence currently exists to inform health professionals in supporting this.

Aims. To describe the prevalence of asthma, asthma management practices and inhalational device use, in a community sample of Australian adults with ID.

Methods. Our retrospective audit combined electronic health records and hard copy records compiled by clinic doctors, to collect information on demographics, living situation, main health conditions and co-morbidities, and access to healthcare. Qualitative data were also collected.

Results. Among the 2254 adults who presented to the clinic, 134 (6%) had doctor diagnosed asthma. Of the 86% who were prescribed asthma medication, all was via the inhalational route. Less than 20% of clients were reported to have been referred to a respiratory specialist, and even less reported to have spirometry performed (3%), or asthma management Plans in place (9%). Nebuliser use was not necessarily consistent with current recommendations. Qualitative analysis revealed inhaler technique and adherence issues.

Discussion. People with ID and asthma are prescribed inhalers. In our study, key indicators of asthma management showed omission of recommended management strategies. In this complex area, there are opportunities for improved respiratory health outcomes for persons with ID, and for pharmacists, as medication experts, to play a more active role.


Effects of three different forms of inhaler technique education for health care professionals on patient asthma outcomes.
Dr Sinthia Bosnic-Anticevich1, Margaret Williamson2, Dr Meg Stuart1, Judith Macksonformerly 2 Biljana Cvetkovski1, Sofia Mavritsakis1, Gosia Mendrela2, Pippa Travers-Mason3, Dr Erica Sainsbury1, Professor Carol Armour4, University of Sydney1; NPS2; Australian Catholic University3, Woolcock Institute of Medical Research4.

Introduction. A high proportion of inhaler device users are not able to do so correctly. A co-ordinated/collaborative approach to asthma management and inhaler device use, could help to address this problem. However, enabling a co-ordinated/collaborative approach is difficult to achieve and establishing evidence of its effect on patient outcomes is a challenge. The Collaborative Asthma Management in the Community (CAMCOM) study evaluated the impact of 3 models of interprofessional education on attitudes towards collaboration and patient health outcomes. This abstract focuses is on patient outcomes.

Aims. To evaluate the effect of three CAMCOM interventions on clinical asthma outcomes.

Methods. HCPs from three general practice networks were recruited into one of three groups (1, 2, and 3) receiving one of three models of inter professional education (joint setting group, online group and socio-cultural theory-based group, respectively). HCPs from a fourth general practice network, received no intervention and acted as a control. Following completion of an educational module, HCPs recruited people with asthma using inhaler devices and provided them with inhaler device education over a six month period (5 visits). Inhaler technique, asthma control, asthma quality of life, perceived control of asthma were evaluated over this time period.

Results. A total of 37 pharmacists, 13 general practitioners and 2 practice nurses recruited 312 patients with asthma. Linear mixed modelling with an autoregressive covariance indicated that there were significant differences between Groups 1, 2, 3 and control over time in terms of inhaler technique, asthma control, asthma quality of life and perceived control of asthma (p<0.0001 for all four outcomes).

Discussion. While the clinical content of continuing profession education sessions can be fixed, the impact of the interaction of health care professionals and the educational material can have a significant impact on the clinical outcomes for people with asthma.
“Knowing how” is not enough: why people with asthma do not maintain correct inhaler technique over time.
Ludmila Ovchinikova, Lorraine Smith, Sinthia Bosnic-Anticevich. The Faculty of Pharmacy, The University of Sydney, Sydney, NSW.

Introduction. Poor inhaler technique maintenance is a persistent problem contributing to suboptimal asthma control. Patient motivation can influence technique maintenance. Motivation is a complex and dynamic phenomenon yet important to understand due to its potential to influence health behaviours. There are no published accounts exploring motivation from the patient’s perspective in relation to inhaler technique maintenance.

Aim. To explore factors that influence motivation for inhaler technique maintenance in people with asthma.

Methods. In-depth semi-structured interviews were conducted with a purposive sample of community dwelling people with asthma from Sydney who had participated in a preceding study involving repeated inhaler technique measures. The interview guide was based on theories of motivation and self-regulation. Interviews were recorded, transcribed verbatim and analysed for themes. Results were validated via independent cross-coding followed by discussion between three researchers. Nvivo 9 software aided data storage and organisation.

Results. 20 interviews were conducted (n=9 “Maintainers” and n=11 “Non-maintainers”) ranging from 25-119 minutes. Multiple factors appeared to influence motivation for inhaler technique maintenance, however three core factors emerged that could sequentially differentiate those participants who maintained correct technique compared to those who did not. They were – 1. perceived threat of asthma, 2. perceived best method of self-management and 3. perceived confidence with self-management. Further, the potential for health care professionals to modify patient perceptions seemed to be strongest at points 2. and 3.

Discussion. In exploring patient motivation and inhaler technique maintenance, three core themes have been uncovered that can characterise those patients who are at greater risk of poor technique maintenance. Further investigation may lead to the development and validation of a potentially useful clinical tool to guide health care professionals in detecting these at risk patients.

Community pharmacy as a health hub: meeting the needs of people with chronic conditions
Sara McMillan1, Amanda J Wheeler1,2, Adem Sav1, Michelle A King3, Jennifer Whitty1, Elizabeth Kendall1, Fiona Kelly1,2. Griffith Health Institute, Griffith University1, Meadowbrook, QLD; Faculty of Medical and Health Sciences, University of Auckland2, Auckland, NZ; School of Pharmacy, Griffith University3, Gold Coast QLD.

Introduction. Pharmacies are frequently visited by people with chronic conditions. There is limited information from the perspective of Australian consumer orientated and professional organisations of consumer needs within the context of community pharmacy.

Aim. To explore the viewpoints of health professional organisations, consumer groups and advocates with respect to the role of community pharmacy in supporting consumers/carers with long term condition(s).

Methods. Semi-structured interviews were conducted with 21 consumer and healthcare stakeholders between January-March 2012; representation was purposively sought from health priority areas defined by the Australian Government. Interviews were conducted face-to-face across the greater Brisbane area and by telephone for others. Interviews were analysed via thematic analysis.

Results. Stakeholders recognised a need for community pharmacy to become a “one-stop” healthcare destination to enable consumers to manage their medications and navigate the health system. Pharmacists were identified as having a “neutral” position and could further develop their health advocacy role. As consumers may not be aware of pharmacists breadth of expertise an improvement and extension of their current role was emphasised.

Discussion. There was a strong perception that community pharmacy practice should shift towards a more patient-centred approach as a health hub destination. This study adds support for pharmacists to collaborate further with consumer support organisations and other health providers to extend their role in chronic illness management.

Grant support: Australian Government Department of Health and Ageing, managed by The Pharmacy Guild of Australia.
**Is the process for approval of high cost drugs for off-formulary use leading to clinically appropriate outcomes?**

Catherine J Lucas¹, Paul Kubler¹, Jennifer H Martin¹²³, Department of Clinical Pharmacology, Royal Brisbane and Women’s Hospital¹, Brisbane, Qld; Department of Internal Medicine, Princess Alexandra Hospital², Brisbane, Qld; The University of Queensland Clinical School Southside³, Brisbane, Qld.

Introduction. Clinical outcome data for off-formulary high cost drug (HCD) use is scant, especially within Australia. Outcome data is important, as use of these drugs is expensive and clinical benefit or detriment is unknown at time of use. A retrospective review of approvals for off-formulary uses of HCD at Royal Brisbane and Women’s Hospital from 2006-2011 was thus performed.

Aims. To analyse clinical outcomes from use of off-formulary drugs for pulmonary hypertension and compare with "expected" outcomes from the literature.

Methods. Data on patients treated for pulmonary hypertension, including brain natriuretic peptide, echocardiography, cardiac MRI, cardiac catheterisation, pulmonary function tests, six minute walk tests, quality of life, WHO functional class and hospital admissions, were collated from HCD approval records, hospital HCD committee meeting minutes, pharmacy dispensing records, patient medical records and pathology and imaging databases. Descriptive statistics on use, as well as clinical outcomes stratified by age, population group and underlying disease compared to "expected outcome" based on population literature for a matched group were undertaken.

Results. Forty-five patients were granted approval for sildenafil, four for iloprost and one each for epoprostenol, prostacycline and tadalafil over six years. We found that there was a poor correlation between clinical outcomes and outcomes expected by the clinical team. Further, there was a large amount of variation in outcomes within a population group.

Discussion. A better methodology for approving HCD is urgently needed. Specifically, the noted poor quality of data provided by the clinical team makes developing a methodology for future studies and regulation important. This data would assist decision-making around HCD use. It would also provide much needed evidence regarding the clinical outcomes and safety of use of newer pharmaceuticals.

**The performance of the Cockcroft-Gault, MDRD and CKD-EPI equations in predicting gentamicin clearance**

Paul K L Chin¹ ², Chris M Florkowski³, Evan J Begg¹², Department of Clinical Pharmacology, Christchurch Hospital¹, NZ. Department of Medicine, University of Otago², Christchurch, NZ. Clinical Biochemistry Unit, Canterbury Health Laboratories³, Christchurch, NZ.

Introduction. Estimating glomerular filtration rate (GFR) is useful for adjusting doses of renally cleared drugs, such as gentamicin.

Aims. To identify the best creatinine-based GFR equation for predicting gentamicin clearance in the context of an isotope dilution mass spectrometry (IDMS)-aligned creatinine assay.

Methods. The bias and imprecision of the Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations for predicting gentamicin clearances, were assessed retrospectively in 255 patients treated with gentamicin during 2011-2012. The local creatinine assay was IDMS-aligned during this period. Gentamicin clearance was calculated using plasma concentrations following dosing with a one-compartment model.

Results. The CG equation had the highest percentage of estimates within 30% of the calculated gentamicin clearance (67%, *P* < 0.001) and lowest root mean square error (39 mL/min) compared with the other two equations, for the entire cohort. Setting a minimum serum creatinine of 60 μmol/L did not improve the performances of the equations. Correction for individual body surface area improved the performances of the MDRD and CKD-EPI equations in the subgroup with body mass indices (BMI) < 18.5 or ≥ 30 kg/m², but not those with BMI 18.5-30 kg/m². The GFR equations performed better in patients with gentamicin clearance < 90 mL/min than those with gentamicin clearance ≥ 90 mL/min; in this latter subgroup, the GFR equations increasingly underestimated gentamicin clearance as gentamicin clearance increased.

Discussion. The Cockcroft-Gault equation provided the best estimate of gentamicin clearance. The CKD-EPI and MDRD equations should be corrected for individual body surface area at the extremes of body size, if used for guiding gentamicin therapy. The GFR equations performed poorly in patients with gentamicin clearance ≥ 90 mL/min.
Describing the use of antibiotics in acute exacerbations of chronic obstructive pulmonary disease (COPD)
Mitchell A McKean. Clinical Pharmacology, Princess Alexandra Hospital, Brisbane, QLD.

Introduction. COPD is a major cause of mortality and significant economic burden within Australia. Clear evidence based guidelines have been developed, however, Australian hospitals have a history of utilising antibiotic regimes that differ from guideline directed therapy.

Aims. To quantify the extent of non-guideline based antibiotic prescribing for patients with acute exacerbations of COPD and identify barriers preventing guideline based antibiotic prescribing.

Methods. A retrospective review of all patients admitted to the Princess Alexandra Hospital (PAH) with a diagnosis of acute exacerbation of COPD over a 6 month period. Patients were excluded if they received non-invasive ventilation, had evidence of pneumonia or sepsis, had an altered level of consciousness or were not for active treatment. Patients were considered in two groups with respect to the antibiotic therapy prescribed: 1) according to guidelines or 2) not according to guidelines.

Results. Of 84 eligible admissions, 6.8% were prescribed antibiotics according to guidelines in the emergency department, while this was the case for 18.3% while inpatients. Patient characteristics did not show a statistically significant difference. The only difference in presenting feature was a significantly higher white cell count in the group prescribed according to guidelines. There was no difference in length of stay or readmission rates at 3 months. There were no complications attributed to prescribed antibiotics and no antibiotic failures.

Discussion. There are clear guidelines that are available in Australia for treating an acute exacerbation of COPD. However, in this tertiary hospital setting, these guidelines are rarely followed. From the data collected, there was no indication of clinical superiority of non-guideline based therapy in terms of duration of hospital admission or readmission rates. Given that there were no apparent benefits of non-guideline based antibiotics, it is difficult to justify.


Is Cytochrome P450 2C19 genotyping cost-effective for guiding clopidogrel treatment in Australia?
Michael J Sorich. Sansom Institute, University of South Australia, Adelaide, SA

Introduction. Cytochrome P450 (CYP) 2C19 genotype has been association with inter-individual variability in the therapeutic effect of the anti-platelet agent clopidogrel. The association is thought to be greatest for individuals undergoing percutaneous coronary intervention (PCI).

Aims. To assess the effectiveness and cost-effectiveness of genotyping CYP2C19 to guide selection of clopidogrel or ticagrelor for individuals with acute coronary syndrome (ACS) and planned PCI.

Methods. A decision-analytic Markov model was designed to enable simulation of the costs, events, and changes in quality-of-life over the lifetime of patients following ACS. Clinical events were derived from the genetic substudy (n=10,285) of the PLATO trial comparing ticagrelor and clopidogrel. Three treatment strategies were assessed: (1) use of clopidogrel for all individuals (‘clopidogrel strategy’), (2) use of clopidogrel for individuals without a CYP2C19 loss-of-function (LoF) allele, and ticagrelor for individuals carrying a CYP2C19 LoF allele (‘genotype strategy’), and (3) use of ticagrelor for all individuals (‘ticagrelor strategy’).

Results. The genotype strategy is more effective than the clopidogrel strategy, and has an incremental cost-effectiveness ratio (ICER) below $20,000 per quality-adjusted life-year (QALY). However, the ticagrelor strategy was found to have the greatest effectiveness and an ICER of less than $30,000 compared to the genotyping strategy. If CYP2C19 gain-of-function (GoF) alleles are also tested, and assuming the effect of a GoF allele is of similar magnitude to that of a LoF allele, then genotyping for CYP2C19 may be the most cost-effective option.

Discussion. Although the genotyping strategy was cost-effective, the ticagrelor strategy is also cost-effective and is the most effective strategy overall. In addition, the genotyping strategy is associated with increased complexity and a point-of-care testing will be required for individuals with ACS. Further research is required to clarify the relative effectiveness of ticagrelor and clopidogrel for individuals carrying a CYP2C19 GoF allele.
Liver Transplant Donor and Recipient CYP3A5 and ABCB1 Genetics and Tacrolimus Pharmacokinetics
Janet K Coller1, Jeyamani Ramachandran2, Alan Wigg2, Matthew Doogue3. Disc Pharmacology, University of Adelaide1; Hepatology, Flinders Medical Centre2; Clinical Pharmacology, Flinders University3, Adelaide, SA.

Introduction. Successful liver transplantation requires immunosuppressant drugs. Tacrolimus, the most widely used, is metabolised by CYP3A enzymes and transported by P-glycoprotein (coded by ABCB1).

Aims. To investigate the impact of genetic variability of recipient and donor CYP3A5 and ABCB1 (P-glycoprotein) on steady state tacrolimus pharmacokinetics and clinical outcomes in liver transplant patients.

Methods. Thirty-two liver transplant recipients were recruited (52 yr; 79% male), and matched donor blood samples were obtained from the Australian Red Cross Blood Service. Both recipients and donors were genotyped for CYP3A5*3 (carriers are non-expressors) and ABCB1 haplotype. Dose-corrected steady state tacrolimus concentrations and the incidences of nephrotoxicity and biopsy proven rejection were compared between genotype groups. Mann-Whitney or Kruskal-Wallis tests, and chi square tests were used for numerical and categorical data, respectively.

Results. Steady state tacrolimus pharmacokinetics were dependent on recipient CYP3A5 genotype: expressors had significantly lower dose-corrected concentrations than non-expressors over all time points, medians 1.15 μg/L and 1.65 μg/L, respectively (P=0.042); but not on donor CYP3A5 expression. In addition, CYP3A5 non-expressor recipients who also had a non-expressor donor liver had the highest concentrations (2.7 μg/L) while the expressor recipient with the expressor donor liver had the lowest concentration (0.94 μg/L) at final follow up (P = 0.023). In contrast, a significant impact of recipient or donor ABCB1 haplotypes on steady-state tacrolimus concentrations was not seen. Differences in CYP3A5 expression or ABCB1 haplotype were not associated with nephrotoxicity or biopsy proven rejection in this small study.

Discussion. Recipient CYP3A5 genotype, present in the gastrointestinal tract, had a significant effect on tacrolimus pharmacokinetics. Donor CYP3A5 genotype, present in the liver, had a lesser effect. This suggests that gastrointestinal wall metabolism is a major determinant of tacrolimus pharmacokinetics. Larger studies are needed to assess the impact of genetic variability in drug transporters on liver transplantation.

Pro-migratory actions of prostacyclin in breast cancer cells that over-express cyclooxygenase-2.
Sarah E Allison, Pei H Cui and Michael Murray. Pharmacogenomics and Drug Development, Faculty of Pharmacy, University of Sydney, NSW 2006, Australia.

Introduction. Metastasis is the major life-threatening characteristic of many tumours. Cyclooxygenase-2 (COX-2) is over-expressed in a range of human tumours and acts in concert with downstream prostaglandin (PG) synthases to generate a series of PG mediators (Wang D & DuBois RN, 2010). While PGE2 has been strongly implicated in metastatic disease the potential roles of alternate PGs are unclear.

Aim. To elucidate the actions of individual PGs on the invasion potential of breast cancer cells that express COX-2.

Methods. The in vitro invasion capacity of MDA-MB-468 breast cancer cells that were engineered to stably express COX-2 was evaluated in 3D-matrigel droplets. All data are n = 3.

Results. Inhibition of PGE2 synthase by CAY10526 (20 μmol/L) decreased cell invasion relative to arachidonic acid alone (20 μmol/L, 79±9 versus 110±9 migrated cells, P<0.05). The prostacyclin synthase inhibitor U-51605 (3 μmol/L) also decreased invasion relative to arachidonic acid treated cells (76±10 versus 110±9, P<0.05). Consistent with the apparent role for prostacyclin, the IP-receptor antagonist CAY10441 decreased invasion (10 μmol/L, 51±9 versus 110±9, P<0.05), although somewhat surprisingly EP-receptor antagonists were ineffective. Inhibitors of PG synthases active in the formation of other prostanoids, and antagonists of alternate prostanoid receptors did not influence tumour cell invasion out of matrigel droplets.

Discussion. These findings implicate prostacyclin in the metastatic activity of MDA-MB-468 breast cancer cells that over-express COX-2. PGE2 also contributed to the invasive properties of the MDA-MB-468 cells but this may be EP-receptor-independent.

46

SPEAKER ABSTRACTS

Anti-proliferative actions of sorafenib and its major metabolites in MDA-MB-231 breast cancer cells

Pei H. Cui, Tristan Rawling, Tina B. Gillani, Xiao-Suo Wang, Fanfan Zhou and Michael Murray. Faculty of Pharmacy, University of Sydney, NSW, Australia.

Introduction. The multi-kinase inhibitor sorafenib is approved for the clinical treatment of renal and hepatic carcinomas and is currently undergoing evaluation for the treatment of breast cancer in combination with other agents. CYP3A4 converts sorafenib to multiple metabolites (Ghassabian et al., 2012) that have been detected in human liver fractions and in patient plasma. The N-oxide (M2) is reported to be an active metabolite, but information on the other metabolites is not currently available.

Aims. This study evaluated the anti-proliferative actions of sorafenib and metabolites (M1-M5) in the MDA-MB-231 breast cancer cell line.

Methods. Sorafenib and its metabolites were synthesised (Ghassabian et al., 2012), and their anti-proliferative actions were evaluated by ATP assay (cell viability), cell cycle kinetics by flow cytometry and western blotting for proteins involved in cell cycle regulation, apoptosis and the MEK/ERK signalling cascade.

Results. Sorafenib and its metabolites (10 μM) decreased the viability of MDA-MB-231 cells and arrested the cell cycle in G0/G1 phase after 24-72 h of treatment. M1-M5 (10 μM) also selectively down-regulated cyclin D1 and E expression, but not cyclin A and B1. Sorafenib and M1-M5 (1 and 10 μM) all strongly down-regulated the expression of Mcl-1 and inhibited the activation of MEK and ERK.

Discussion. Sorafenib undergoes oxidation to several active metabolites (M1-M5) with the potential to impair the growth of breast cancer cells. By targeting the anti-apoptotic Mcl-1 and growth stimulatory MEK/ERK pathways these metabolites may contribute to the actions of sorafenib in breast cancer.

Differential effects of mango peel sub-fractions on lipid accumulation in 3T3-L1 adipocyte cells
Meng-Wong Taing¹, Jean-Thomas Pierson¹, Paul N. Shaw¹, Ralf G. Dietzgen¹,², Sarah J. Roberts-Thomson¹, Michael J. Gidley¹,² and Gregory R. Monteith¹. School of Pharmacy, The University of Queensland¹, QLD; Queensland Alliance for Agriculture and Food Innovation, Centre for Nutrition and Food Sciences, The University of Queensland², St Lucia, QLD.

Introduction. Plant phytochemicals represent a class of bioactive molecules that may be beneficial for human health. Recent research in mangoes suggests that extracts from different cultivars can inhibit adipogenesis in the 3T3-L1 adipocyte cell line. Mango peel is reported to have greater bioactive effects on adipogenesis than does mango flesh. Aim. In this study, peel extracts from cultivars Irwin (IW), Nam Doc Mai (NDM) and Kensington Pride (KP) were separated into four fractions and assessed for their effects on lipid accumulation in differentiating 3T3-L1 cells.

Methods. Mango peel methanol extracts were separated into four fractions using preparative HPLC. Fraction 1 contained the most hydrophilic components whilst subsequent fractions contained increasingly more hydrophobic components. 3T3-L1 pre-adipocytes were induced to differentiate with or without mango peel extract fractions (at 1, 10, 30, 100 μg/mL) for seven days. High content imaging was used to assess lipid accumulation at day seven. Mass spectrometry was used to identify unique compounds in mango peel fractions.

Results. For the three mango cultivars, the more hydrophilic peel fractions 1-3 inhibited lipid accumulation with greater potency than the more hydrophobic peel fraction 4. Fractions 1-3 displayed biphasic effects on lipid accumulation whereas fraction 4 displayed variable bioactive effects on lipid accumulation. From all cultivars, the more lipophilic fraction 4 enhanced lipid accumulation greater than fractions 1-3. Using mass spectrometry, five long chain free fatty acids were detected in fraction 4 that were not present in peel fractions 1-3. IW fraction 4 contained the highest level of free fatty acids compared to other mango cultivars, an observation consistent with its ability to promote rather than inhibit lipid accumulation at high concentrations.

Discussion. Fatty acids present within mango peel extracts may be responsible for the bioactive differences observed between different mango cultivars on lipid accumulation in 3T3-L1 cells.

The preparation and characterisation of polypyrrole particles for tuneable drug delivery
Zaid Aqrawe¹, Emeel Farjan¹, David Ting¹, Brett Tomkins¹, Kevin Xu¹, Darren Svirskis¹, Manisha Sharma¹, Zimei Wu¹. School of Pharmacy, Univ of Auckland¹, Auckland, NZ. (Introduced by Maree Jensen, Univ of Auckland, Auckland, NZ)

Introduction: Polypyrrole (PPy) is an intrinsically conducting polymer. This electroactive polymer responds to electrical stimulus and can be used in tuneable drug delivery systems. PPy particles can be prepared from soft templates including micelles and microemulsions. The model compound used in this study is risperidone, a poorly water soluble, antipsychotic drug.

Aims: To prepare and characterise the physiochemical properties of PPy particles loaded with risperidone.

Methods: Micelles and microemulsions were used as soft templates to synthesise PPy particles. Risperidone was dissolved in the template and incorporated into PPy particles during the interfacial polymerisation of pyrrole. Drug loading and entrapment efficiencies were determined by HPLC, morphology was assessed by SEM and electroactivity explored through cyclic voltammetry. Electrically triggered in-vitro release studies were also carried out.

Results: PPy particles loaded with risperidone were produced using soft template methods. Micellar methods yielded an entrapment efficiency of 32.5 ± 9.8% with a drug loading value of 3.5 ± 0.6%, w/w. The microemulsion system yielded an entrapment efficiency of 52.2 ± 7.5% with a drug loading of 3.2 ± 0.5%, w/w. Cyclic voltammetry demonstrated the electroactivity of the PPy particles and in-vitro release studies showed electrically tuneable risperidone release.

Discussion: The entrapment efficiencies of the micellar systems remained relatively constant when different risperidone concentrations were used during preparation, indicating higher drug loading could be achieved when higher drug concentrations were used. In contrast, for the particles formed from microemulsions, higher drug concentrations during preparation did not increase drug loading as the entrapment efficiency reduced proportionately. The demonstrated electroactivity of the particles makes them promising candidates for tuneable drug delivery systems.
**Characterisation of emulsions as potential saliva substitutes in xerostomia**

Sara M. Hanning¹, Tao Yu², David S. Jones², Jules A. Kieser³, Natalie J. Medlicott¹. School of Pharmacy, Univ of Otago¹, Dunedin, NZ; School of Pharmacy Queen’s Univ of Belfast², Belfast, NI; Sir John Walsh Research Institute³, Univ of Otago, Dunedin NZ.

Introduction: Xerostomia is a condition involving reduction or absence of saliva, causing difficulties swallowing, speaking and eating, and an increased incidence of dental caries and periodontal disease (Jensen et al, 2010). Current treatments fail to provide adequate long-term relief, particularly in patients with minimal salivary gland function.

Aim: To investigate the physicochemical properties of emulsions for use as saliva substitutes.

Methods: Rice bran oil (RBO), lecithin:propylene glycol (1:1 w/w, SM) and water were used to prepare compositions in a pseudoternary phase diagram. These were assessed by polarising light microscopy (MoticBA300, Hong Kong), flow and oscillatory rheology (TA Instruments, England). The rheometer was fitted with a 40mm parallel-plate and solvent trap. Shear rate was 0–50s⁻¹ for flow rheology, oscillation was 0.1–10Hz for oscillatory rheology and temperature was 37°C. Storage (G’) and loss (G”) moduli were used to calculate tanδ (G”/G’).

Selected compositions were analysed for droplet changes with frequency using a small-angle light scattering (SALS) accessory.

Results: A small microemulsion and large liquid crystalline region were observed. Compositions with greater than 30% SM exhibited pseudoplastic flow, with apparent viscosity increasing with SM concentration. At 20-30% SM, tanδ was greater than one at frequencies below 5Hz and less than one at higher frequencies. A peak in tanδ was observed in formulations containing over 30% SM. SALS demonstrated the frequency of this peak (range: 2.4–8.7Hz) coincided with an increase in light intensity, suggesting a reversible increase in droplet size.

Discussion: Properties depended upon composition, shear rate and oscillatory frequency, which are important considerations in the oral environment. Frequency-dependent structure may be useful if liquid-like properties (tanδ>1) dominating at low frequencies assist lubrication of the oral cavity at rest, whereas solid-like properties (tanδ<1) assist in retention during high-frequency tasks such as chewing and swallowing.


**Conformational stability of various proteins in solid lipid matrices prepared by melting and cooling**

Farrukh Zeeshan¹, Lene Jorgensen², Natalie J Medlicott¹. School of Pharmacy, University of Otago¹, Dunedin, New Zealand; Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen², Denmark.

Introduction. Protein drugs are an important class of therapeutics (Antonio et al, 2007). Lipid can be used to prepare controlled release dosage forms without organic solvents (Walduck et al, 1998). However, protein may degrade by heat used to melt the lipid (Riethmier et al, 2001).

Aim. To understand conformational stability of proteins in lipid matrices prepared by melting and cooling.

Methods. Bovine serum albumin (BSA), lysozyme (LZ), horse radish peroxidase (HRP) and catalase (CT) were used. Each was heated alone at 80 0 and 140 0C and with Precirol AT05 (glycerol palmitostearate, melting point 58°C) at 80 0C up to 96hrs. Aliquots were cooled to room temperature and analysed using ATR. Proteins were dissolved in water and enzyme activity and SEC determined.

Results. Heating solid protein to 80ºC did not alter the physical appearance whereas heating to 140°C resulted in a colour change and reduced aqueous solubility. Enzyme activity of LZ, HRP and CT was lost while LZ was reduced to 35%. SEC showed 63% BSA and 82% LZ remained following heating of protein solid at 80°C. ATR spectra of the amide I band of heated protein (80ºC and 140ºC) were not significantly different to the control non heat-exposed protein. In the presence of lipid CT showed the greatest change in secondary structure.

Discussion. ATR spectroscopy could not predict loss of activity of enzymes exposed to heat in the solid state. In lipid, change in protein secondary structure was observed for CT which represented the most heat labile model protein.


PEGylated surfactants inhibit the digestion of co-formulated triglycerides in a PEG chain length dependent manner.

Orlagh M Feeney¹, Hywel D Williams¹, Colin W Pouton², Christopher J H Porter¹. Drug Delivery Disposition and Dynamics¹, Drug Delivery Biology², Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Melbourne, VIC.

Introduction. Lipid-based formulations (LBFs) can improve the oral absorption of poorly water-soluble drugs by delivering the drug to the gastro-intestinal tract in a pre-dissolved, molecularly dispersed form. However, on entering the intestine, LBFs are rapidly digested by intestinal lipases, causing a digestion rate dependent loss in solubilisation capacity, in turn creating the risk of drug precipitation and reduced bioavailability. Surfactants containing polyethylene glycol (PEG) hydrophilic headgroups have previously been suggested to inhibit the digestion of medium-chain triglycerides (MCT).

Aims. To evaluate the effect of six classes of PEGylated surfactant on the in vitro digestion of medium-chain triglycerides

Methods. An in-vitro digestion model was used to examine the influence of PEGylated surfactants on the digestion of MCT. The time required for 10% of the MCT to be digested (T10%) was estimated by linear interpolation and plotted as a function of the chain length of the PEG surfactant component.

Results. The presence of PEGylated surfactants altered the digestion of co-formulated MCT. Surfactants containing short and long PEG chains were poor inhibitors of digestion. However for intermediate PEG-chain length surfactants, digestion inhibition, presumably by formation of a PEG mantle around the lipidic micellar core, was effective in increasing the time for 10% of MCT to digest.

Discussion. PEGylated surfactants are effective in modulating MCT digestion rate. The potential to delay digestion mediated changes in solubilisation capacity may provide an improved platform for oral lip based drug delivery.

The lymphatic system is critical in maintaining the prolonged circulation of monoclonal antibodies and in promoting absorption from the subcutaneous injection site

Annette M Dahlberg, Lisa M Kaminskas, Michelle P McIntosh, Juergen Bulitta, Joseph Nicolazzo. Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC.

Introduction. Therapeutic monoclonal antibodies are currently delivered IV, although SC administration is a therapeutic goal. Thus far, however, little is known about the mechanisms by which antibodies are absorbed from subcutaneous injection sites, and the role of the lymphatic system in their absorption and IV PK.

Aims. To characterise the role of the lymphatic system in the absorption of a model monoclonal antibody (trastuzumab) from SC injection sites and in its IV PK.

Methods. The PK and lymphatic uptake of trastuzumab were examined in rats after IV and SC dosing via ELISA. A population PK model was developed and fitted to the data.

Results. The bioavailability of trastuzumab in rats was approximately 80% after SC administration and the antibody displayed a prolonged circulatory half life of 2 weeks following IV and SC administration in non-lymph cannulated, control rats. Plasma concentrations in lymph cannulated animals, however, were significantly lower than in control animals and approximately 44% and 27% of the administered dose was recovered in thoracic lymph over 30 h after IV and SC administration respectively (see figure). A population PK model was developed based on the data to predict the long term PK behaviour of trastuzumab in rats.

Discussion. This study highlights for the first time the significant role of the lymphatic system in maintaining the long circulatory behaviour of trastuzumab. The results of this study also show that the lymphatic system is a major pathway by which monoclonal antibodies are absorbed from SC injection sites.
Understanding the role of device design on the aerosolisation of a carrier-based dry powder inhaler
Qi Tony Zhou1, Zhenbo Tong2, Patricia Tang1, Runyu Yang2, Hak-Kim Chan1. 1: Advanced Drug Delivery Group, Faculty of Pharmacy, University of Sydney, Sydney, Australia; 2: Laboratory for Simulation and Modelling of Particulate Systems, School of Materials Science and Engineering, University of New South Wales, Sydney, Australia

Introduction. The design of inhaler devices plays a critical role on the aerosolisation of the DPI formulations. The device design affects the drug detachment from carriers for the carrier-based formulations. Computational fluid dynamics (CFD) analysis is useful to simulate the flowfield generated in the device and thus to elucidate the mechanism of the effect of device design on the de-agglomeration.

Aims. To investigate experimentally the effect of device design on the aerosol performance of carrier-based DPI systems coupled with CFD analysis.

Methods. A commercial Aerolizer® device was modified with different geometries – air inlet, mouthpiece and grid. The in vitro aerosol performance was evaluated by a multi-stage liquid impinger. CFD analysis was performed to investigate the air flow pattern inside the inhaler devices.

Results. The aerosol fine particle fraction (FFP) below 5 μm was significantly improved with the reduced inlet size, attributed to the increased air velocity from CFD analysis. No significant differences were shown in the FFP with the modified mouthpiece and grid, except more drugs deposited in mouthpiece and throat as the grid voidage was increased.

Discussion. Air inlet and grid designs are critical to the aerosolisation of carrier-based DPIs. CFD analysis is useful in understanding the effect of device design.
Impact of Alzheimer’s disease on drug transport across the blood-brain barrier
Dharmini C Mehta¹, Jennifer L Short², and Joseph A Nicolazzo¹. ¹Drug Delivery, Disposition and Dynamics, ²Drug Discovery Biology, MIPS, Monash University, Parkville, VIC

Introduction: There are various blood-brain barrier (BBB) related pathological changes reported in Alzheimer’s disease (AD) but less is known about what impact such alterations have on the ability of therapeutic agents to enter the brain.

Aim: The aim of this study was to systematically assess the BBB transport of probe compounds in a relevant animal model of AD.

Methods: ³H- and ¹⁴C-labelled compounds were perfused into the brain of 3×Tg (TG) and wild type (WT) AD mice by transcardiac perfusion. After a 4 min perfusion, cortex, hippocampus and perfusate concentrations were determined by liquid scintillation counting, and cortex/hippocampus-to-perfusate ratios (C:P/H:P) calculated. To understand the observed in vivo transport alterations, molecular characterization of the BBB was performed, with collagen-IV and P-glycoprotein (P-gp) expression assessed by immunohistochemistry and western blot, respectively.

Results: C:P and H:P ratio of the paracellular marker ¹⁴C-sucrose was not significantly different between WT and TG mice. BBB transport of the passive diffusion markers ³H-diazepam and ³H-propranolol were significantly (p<0.05) decreased by 54-60% in TG mice relative to WT mice, whereas the BBB transport of P-gp substrates (³H-digoxin, ³H-loperamide and ³H-verapamil) was not different between genotypes. There was significant thickening of the basement membrane as observed by a 33% increase in collagen-IV staining in brain slices of TG mice, and a 20% reduction in P-gp expression in the isolated microvessels of TG mice.

Discussion: Consistent with that observed clinically, the BBB paracellular route is maintained in TG mice. The BBB transport of passively-diffusing compounds is reduced in TG mice likely as a result of increased cerebrovascular membrane thickness. In contrast, the BBB transport of P-gp substrates appears unaffected in TG mice, as the reduced expression of P-gp is likely compensated by a thickened basement membrane. These studies suggest that AD significantly alters disposition of therapeutics into the brain.

Contributions of rCtr1 to the uptake and toxicity of copper and platinum anticancer drugs in sensory neurons
Johnson J Liu¹ ², Yaeseul Kim², Fang Yan², Qi Ding², Julian Mercer³, Mark J McKeage³. School of Pharmacy, Faculty of Health Science, Univ of Tasmania¹, Hobart, Tasmania; Dept of Pharmacol & Clin Pharmacol, School of Medical Sciences, Faculty of Medical and Health Sciences, Univ of Auckland², Auckland; Research Centre for Cellular and Molecular Biology; School of Life and Environmental Sciences, Deakin Univ³, Victoria

Introduction. Dorsal root ganglion (DRG) neurons are affected by platinum anticancer drug-induced neurotoxicity and neurodegenerative processes associated with disturbed copper homeostasis and transport.

Aims. To understand the role and functional activity of rat copper transporter 1 (rCtr1, Slc31a1) in the uptake and toxicity of copper and platinum drugs in cultured rat DRG neurons.

Methods. Recombinant rCtr1-overexpressing cell lines and cultured rat DRG neurons were studied by methods using ICP-MS, MTT assay, immunocytochemistry and RT-PCR.

Results. Heterologous expression of rCtr1 in HEK293 cells (HEK/rCtr1 cells) increased the uptake and cytotoxicity of copper, oxaliplatin, cisplatin and carboplatin, in comparison to isogenic vector-transfected control cells. Cultured rat DRG neurons endogenously expressed rCtr1 protein on their neuronal cell body plasma membranes and cytoplasm, and displayed substantial capacity for taking up copper, but were resistant to copper toxicity. The uptake of copper by both cultured rat DRG neurons and HEK/rCtr1 cells was saturable and inhibited by cold temperature, silver and zinc, consistent with it being mediated by rCtr1. Cultured rat DRG neurons accumulated platinum during their exposure to oxaliplatin and were sensitive to oxaliplatin cytotoxicity. The accumulation of platinum by both cultured rat DRG neurons and HEK/rCtr1 cells, during oxaliplatin exposure, was saturable and temperature dependent, but was inhibited by copper only in HEK/rCtr1 cells.

Discussion. rCtr1 can transport copper and platinum drugs, and sensitizes cells to their cytotoxicities. DRG neurons display substantial capacity for taking up copper via a transport process mediated by rCtr1, but appear able to resist copper toxicity and use alternative mechanisms to take up oxaliplatin. Supported by grants from Cancer Society of New Zealand, Faculty Research Development Fund and NM McBeath Child Cancer Fund.
PEGylation improves the lymphatic disposition of interferon 2α after subcutaneous and intravenous administration in rats and consequently improves the treatment of lymph-metastatic breast cancer

Lisa M Kaminskas1, Jurgen Bulitta1, Victoria McLeod1, Caroline Lee2, Erica K Sloan2, Christopher JH Porter1. Drug Delivery Disposition and Dynamics2; Drug Discovery Biology2, Monash Institute of Pharmaceutical Sciences (MIPS), Melbourne, VIC.

Introduction. The lack of appropriate lymph-targeted medications has thus far hindered the successful treatment of lymph-resident diseases. For instance, protein-based immunomodulators such as interferon and interleukin have potential in the treatment of lymphatic cancers, yet they display limited lymphatic access and efficacy.

Aims. This work aimed to examine whether PEGylation improves the lymphatic disposition of interferon 2α and whether this translates into improved chemotherapeutic efficacy against lymph-metastatic breast cancer.

Methods. The lymphatic pharmacokinetics of native interferon 2α (Intron A®, 19 kDa) and PEGylated interferon (PEG-Intron®, 31 kDa; PEGASYS®, 60 kDa) were examined in rats. The chemotherapeutic efficacy of SC Intron A and PEG-Intron were compared in mice bearing axillary lymph node MDA MB-231 metastases.

Results. Intron A was poorly absorbed from the SC injection site (F abs 36%) and showed little uptake into lymph after SC or IV administration (≤1%). In contrast, PEG-Intron displayed better absorption from the SC injection site (F abs 82%) and lymphatic access after SC (20%) and IV (8%) dosing. PEGASYS, however, was incompletely absorbed from the SC injection site (F abs 23%) and showed similar lymphatic access after SC administration to PEG-Intron (21%). The lymphatic disposition of PEGASYS after IV administration, however, was significantly greater (29%) when compared to IV PEG-Intron. SC administration of Intron A below the 3rd mammary fat pad in mice bearing axillary breast cancer metastases, or SC administration of PEG-Intron on the opposite side to axillary tumour growth had no chemotherapeutic effect. SC administration of PEG-Intron on the same side as the axillary metastasis, however, inhibited tumour growth.

Discussion. PEGylation has the potential to improve the lymphatic disposition and in vivo efficacy of small therapeutic proteins with indications in lymphatic diseases. PEG molecular weight and loading, however, need to be optimised for each protein to maximise absorption from the injection site, lymphatic access and receptor binding affinity – all critical determinants of therapeutic success.

Association between intra-renal P-gp expression and cyclosporine concentrations in renal transplantation.

Benedetta C Sallustio1,2, Benjamin D Noll1, Janet K Coller2, Andrew A Somogyi2. Dept of Clin Pharmacol, The Queen Elizabeth Hosp1, Woodville, SA; Discipline of Pharmacol, The Univ of Adelaide2, Adelaide, SA.

Introduction. Although immunosuppression with calcineurin inhibitors (cyclosporine, tacrolimus) has significantly improved graft survival in renal transplantation, their long-term use is limited by nephrotoxicity. Cyclosporine and tacrolimus are substrates for the membrane efflux transporter P-glycoprotein (P-gp), and P-gp expression has been identified as a significant determinant of chronic tubulointerstitial damage in transplanted kidneys (Naesens et al, 2009). Graft P-gp expression is likely to play a role in limiting intra-renal accumulation (hence toxicity) of calcineurin inhibitors. However the relationship between P-gp expression and graft calcineurin inhibitor concentrations has not been investigated.

Aims. To determine the association between renal proximal tubular P-gp expression and graft cyclosporine concentrations in renal transplant recipients.

Methods. This was a retrospective study in 35 transplant recipients for whom routine biopsies had been performed with 1 month post-transplantation. For each subject, P-gp expression was assessed by immunohistochemistry in two paraffin-embedded biopsy samples, one taken pre-perfusion and the other within 1 month post-perfusion. Subjects were categorised by whether there was decreased, unchanged or increased P-gp expression in the post- versus pre-perfusion sample. Graft cyclosporine concentrations were measured in matching post-perfusion frozen biopsy samples by LC-MS/MS (Noll et al, 2011). Corresponding whole blood cyclosporine concentrations (2 hr post dose) were collected from therapeutic drug monitoring records.

Results. There was no correlation between cyclosporine concentrations in blood and renal tissue (r=0.31, P=0.07). There was no significant effect of P-gp expression on blood cyclosporine concentrations (one-way ANOVA P=0.12). However, there was a significant association between intra-renal P-gp expression and renal cyclosporine concentrations (one-way ANOVA), with mean±SD concentrations of 8.8±4.2 and 3.8±3.9 ng/mg tissue (P=0.04) in grafts with decreased (n=10) or increased (n=6) P-gp expression, respectively.

Discussion. These observations suggest that, despite therapeutic drug monitoring to minimise inter-individual variability in systemic cyclosporine concentrations, graft P-gp expression significantly determines local cyclosporine exposure.

Noll B et al (2011) Ther Drug Monit 33:688-693
Population pharmacokinetic modelling of colistin methanesulphonate and formed colistin in patients on continuous ambulatory peritoneal dialysis
Cornelia B Landersdorfer¹, P Koomanachai², G Chen³, Hee-Ji Lee³, Jovan Jacob³, A Jitmuang², S Wasuwattakul², S Sritippayawan², Jian Li¹, Roger L Nation³, Visanu Thamlikitkul². Centre for Medicine Use and Safety, Monash University¹, Parkville, VIC; Faculty of Medicine Siriraj Hospital, Mahidol University², Bangkok, Thailand; Monash Institute of Pharmaceutical Sciences, Monash University³, Parkville, VIC.

Introduction. Colistin, administered intravenously as its inactive prodrug colistin methanesulphonate (CMS), is increasingly used as last-line therapy to combat multidrug-resistant gram-negative bacteria. CMS dosing needs to be adjusted for renal function. The impact of continuous ambulatory peritoneal dialysis (CAPD) on the pharmacokinetics of both CMS and colistin formed from it in the body has not been studied.

Aims. To quantitatively describe and predict the time course of CMS and formed colistin simultaneously in plasma and dialysate of CAPD patients.

Methods. Eight CAPD patients received a single iv CMS dose (150 mg colistin base activity) over 30 min. Serial blood and dialysate samples were collected over 24 h, and cumulative urine where applicable. Concentrations were determined by HPLC. Population modelling was performed in S-ADAPT.

Results. A model with two disposition compartments for CMS, one for colistin, and first-order formation of colistin from CMS well described all data simultaneously. Total body clearance of CMS (excluding CAPD clearance) was 1.77 L/h (44%) [population mean (between subject variability)], while CAPD clearance was 0.088 L/h (64%). The population mean terminal half-life of CMS was 8.4 h. For colistin, total clearance/fm (excluding CAPD clearance; fm, fraction of CMS metabolised to colistin) was 2.74 L/h (50%), CAPD clearance was 0.101 L/h (34%), and mean terminal half-life 13.2 h. Including conversion of CMS to colistin in dialysate in the model allowed adequate description of the time courses of CMS and colistin in dialysate.

Discussion. Clearances by CAPD were low for both CMS and formed colistin. This suggests that doses should not be increased during CAPD. This model can be used to predict colistin concentrations and target attainment in CAPD patients for other than the studied dosage regimen and to optimise CMS dosage regimens in CAPD patients.

Development of a population model of early rheumatoid arthritis disease progression treated with methotrexate, sulfasalazine and hydroxychloroquine.
Jessica Wojciechowski¹, Richard N. Upton¹, Michael D. Wiese², David J. R. Foster¹. Australian Centre for Pharmacometrics, School of Pharm and Med Sci, UniSA, Adelaide, SA¹. Sansom Institute for Health Research, UniSA, Adelaide, SA².

Introduction. Identification of markers that predict the rate and extent of rheumatoid arthritis progression could lead to improved guidelines for disease management and individualised treatment strategies.

Aim. To develop a disease progression model for early rheumatoid arthritis, identify factors affecting DMARD response, and apply Bayesian estimation methods to forecast an individual’s response.

Methods. A population disease progression model for early rheumatoid arthritis was developed using NONMEM and DAS28 obtained from 263 patients who attended the Royal Adelaide Hospital from initiation of therapy (consisting of methotrexate, sulfasalazine and hydroxychloroquine) until 60-weeks. Various base models and covariates were analysed to describe disease progression for the average individual in the population, and subpopulations, respectively. Bayesian estimation was performed in NONMEM using the model and known DAS28 samples.

Results. An exponential model, additive to baseline DAS28, with covariance between parameters, and combined residual error model was developed. The population estimates from the final model were baseline DAS28 (5.62), extent (-1.28 DAS28 units) and rate of disease progression (-0.109 DAS28 units/week). Older individuals exhibited more severe baseline DAS28, those with more severe baseline disease activity received corticosteroids, and current/past smokers achieved 79% of the extent of non-smokers’ response. Data rich sample schedules were needed to accurately estimate an individual’s future time-course of disease progression.

Discussion. This is the first report of a disease progression model for early rheumatoid arthritis. Bayesian estimation demonstrated potential to evaluate the number, and the interval of DAS28 required to accurately forecast response.
Evaluation of current warfarin pharmacokinetic-pharmacodynamic models and dosing algorithms.
Verity Pearson-Dennett¹, Michael B Ward¹, David JR Foster¹. Australian Centre of Pharmacometrics, School of Pharm and Med Sci, UniSA, Adelaide, S.A.

Introduction. Warfarin therapy is complicated by wide inter-individual variation in response, due to polymorphisms impacting pharmacokinetics (CYP2C9) and pharmacodynamics (VKORC1). Genetically-guided (PGx) dosing algorithms have been developed based in part upon population PK-PD models, to reduce the need for INR monitoring. These typically over- emphasise the performance for the ‘average’ patient, and have not reported the range of outcomes expected in the population.

Aims. To investigate the application of PGx dosing algorithms without INR supervision on INR outcomes and compare INR profiles with current clinical practice.

Methods. 1000 patients representative of a Caucasian population (age, weight and height) were obtained using P3M software. Using the NONMEM and R software, Monte-Carlo simulations of a PGx algorithm (Avery et al., 2011) and current clinical practice (Roberts et al., 2003) were performed using a published PK/PD model (Hamberg et al., 2010) to describe INR profiles.

Results. With the unsupervised PGx algorithm, the least sensitive genotype (CYP2C9*1/*1, VKORC1 G/G) reaches an average INR of 2. The most sensitive (CYP2C9*3/*3, VKORC1 A/A) genotype reaches an average INR of 2, with a maximum INR value of 7.5. Adjusting doses according to age and INR markedly decreases inter-individual variability (IIV).

Discussion. PGx dosing provides good estimates of doses, but without monitoring results in some patients attaining dangerous INRs. Adjusting doses in response to INR reduces variability, but provides inappropriate dosing decisions for warfarin sensitive patients. These results highlight the need for ongoing INR monitoring and incorporating PGx guided dose adjustment protocols.


Development and application of a mechanism-based model for the multistage life-cycle of murine malaria - the effect of single and multiple dose dihydroartemisinin
Kashyap Patel¹, Kevin T Batty², Brioni R Moore³, Peter L Gibbons²,⁵, Jürgen B Bulitta¹ & Carl M Kirkpatrick¹. Centre for Medicine Use and Safety, Monash Univ¹, Melbourne, VIC; School of Pharmacy, Curtin Univ², Bentley, WA; Curtin Health Innovation Research Inst, Curtin Univ³, Bentley, WA; School of Medicine and Pharmacology, Univ of Western Australia⁴, Crawley, WA; Dept of Medical Technology & Physics, Sir Charles Gardiner Hospital⁵, Nedlands, WA.

Introduction. Murine models are used to study erythrocytic stages of malaria infection, because parasite morphology and development are comparable to human malaria infections. However, mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) models for antimalarials are scarce, despite their potential to optimize antimalarial combination therapy.

Aims. To develop a mechanism-based growth model (MBGM) for P. berghei, and then characterize the parasiticidal effect of dihydroartemisinin (DHA) in murine malaria (MBGM-PK-PD).

Methods. Stage-specific (ring, early trophozoite, late trophozoite and schizont) parasite density data from Swiss mice inoculated with Plasmodium berghei were used for model development in S-ADAPT. A single intraperitoneal dose of DHA (10–100 mg/kg) or vehicle was administered at 56 h after inoculation. In an independent study, a multiple-dose regimen of DHA (10 mg/kg per dose) was administered at 0, 12, 24, 36 and 48 h post-inoculation.

Results. The MBGM explicitly reflected all four erythrocytic stages of the P. berghei life-cycle. Merozoite invasion of erythrocytes was described by a first-order process that declined with increasing parasitaemia. A 1-compartment model with zero order absorption described the PK of DHA, with an estimated clearance and distribution volume of 1.95 L h⁻¹ and 0.851 L, respectively. Parasite killing was described by a turnover model, with DHA inhibiting the production of physiological intermediates (IC₅₀ 1.46 ng/mL). The same structural model adequately described the parasiticidal effect of DHA after multiple dosing over 5 days.

Discussion. Overall, the MBGM-PK-PD described the rise in parasitaemia, the nadir following DHA dosing, and subsequent parasite resurgence. This novel model is a promising tool to study malaria infections, identify the stage-specificity of antimalarials and provide insight into antimalarial treatment strategies.
Renal function estimation in drug development: should East Asian ethnicity be considered?
Daniel Trajkov1, Sophie L Stocker2, Annette S Gross1,2. Faculty of Pharmacy, Univ of Sydney1, Sydney, NSW; Ethnopharmacology, GlaxoSmithKline R&D2, Ermington, NSW.

Introduction. The growing globalisation of clinical drug development warrants consideration of the influence of ethnicity on drug pharmacokinetics, efficacy and safety. Ethnic-specific GFR-estimating equations have been developed for East Asian populations to assess renal function, a factor which can influence drug pharmacokinetics. However, the implications of using, or ignoring, East Asian-specific GFR-estimating equations in drug development programs have not yet been reported.

Aims. To evaluate the consequences of using East Asian-specific equations to assess renal function in subjects of East Asian ancestry relative to using conventional equations.

Methods. Baseline demographic data (age, sex, ethnicity, weight, serum creatinine concentration) were obtained from ethnically diverse cohorts of subjects enrolled in 17 clinical studies (including healthy subjects, subjects with renal impairment, type 2 diabetes, benign prostatic hyperplasia or renal cell carcinoma). Creatinine clearance (CrCL) was estimated in each subject using the Cockcroft-Gault (CG) equation and GFR was estimated using the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations and the ethnic-specific Chinese (C-MDRD), Japanese (J-MDRD) or Korean (K-MDRD) MDRD equations or the Japanese CKD-EPI (J-CKD-EPI) equation.

Results. Subject demographics and renal function estimates (mean±SEM) calculated using the conventional and East Asian-specific equations are given in the table.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>N</th>
<th>Age (years) [range]</th>
<th>Sex (% male)</th>
<th>CrCL (mL/min)</th>
<th>Estimated GFR (mL/min/1.73m2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CG</td>
</tr>
<tr>
<td>Caucasian</td>
<td>1049</td>
<td>60±0.3 [17-90]</td>
<td>61</td>
<td>91±1.2</td>
<td>84±0.9</td>
</tr>
<tr>
<td>Chinese</td>
<td>612</td>
<td>55±0.5 [19-80]</td>
<td>50</td>
<td>91±1.2</td>
<td>98±1.2</td>
</tr>
<tr>
<td>Japanese</td>
<td>211</td>
<td>55±1.2 [21-83]</td>
<td>70</td>
<td>95±2.2</td>
<td>113±1.7</td>
</tr>
<tr>
<td>Korean</td>
<td>56</td>
<td>63±1.1 [41-83]</td>
<td>91</td>
<td>65±2.7</td>
<td>71±2.2</td>
</tr>
</tbody>
</table>

Discussion. In East Asian subjects, renal function estimates differ when determined by ethnic-specific and conventional GFR-estimating equations. Further evaluation of East Asian-specific GFR-estimating equations is warranted to determine the implications of the differences in estimated renal function for drug development and clinical practice.

The population pharmacokinetics of allopurinol and oxypurinol.
Daniel FB Wright1, Lisa K Stamp2, Tony R Merriman1, Murray L Barclay2, Stephen B Duffull1, Nicholas HG Holford4. Sch of Pharm, Uni of Otago1, Dunedin, NZ; Depart of Med, Uni of Otago2, Christchurch, NZ; Depart of Biochem, Uni of Otago3, Dunedin, NZ; Depart of Pharmacol and Clin Pharmacol, Uni of Auckland4, Auckland, NZ.

Introduction. Allopurinol is used to treat gout and works by reducing serum urate concentrations. Dose requirements are highly variable between patients and there is currently no satisfactory means of predicting the maintenance dose required to achieve the serum urate target of <0.36mmol/L. A better understanding of the factors which determine the variability in allopurinol and oxypurinol pharmacokinetics between patients is required.

Aims. To develop a population pharmacokinetic model for allopurinol and oxypurinol, and, to explore the influence of patient characteristics on allopurinol and oxypurinol pharmacokinetics.

Methods: A population analysis was carried out using NONMEM. A total of 680 allopurinol and 694 oxypurinol plasma concentrations (n=104) were available for analysis. The effects of renal function, body composition, drug interactions, and genetic variability in renal urate transporters were evaluated.

Results. A parent-metabolite model with a two compartment model for allopurinol and a one compartment model for oxypurinol was fitted to the data. Renal function, fat-free mass (FFM) and diuretic use were found to predict differences in the pharmacokinetics of oxypurinol. The population estimates for allopurinol clearance, central and peripheral volume and inter-compartmental clearance were 50 L/h/70 kg FFM, 11.4 L/70 kg FFM, 91 L/70 kg FFM and 142 L/h/70 kg FFM respectively with a between subject variability of 33% coefficient of variation (CV) for allopurinol clearance. Oxypurinol clearance and volume of distribution were estimated to be 0.78 L/h/70 kg FFM for a patient with a creatinine clearance of 100 mL/min and 41 L/70 kg FFM with a between subject variability of 28% and 15% (CV) respectively.

Discussion. This research represents a step towards a pharmacokinetic-pharmacodynamic and disease progression model for the relationship between allopurinol dose, serum urate concentration and clinical outcome. Future work will explore the probability of successfully achieving serum urate targets in patients with impaired renal function.
**162**

**The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin**

Sophie L Stocker1, Kari M Morrissey1, Sook Wah Yee1, Richard A Castro1, Lu Xu1, Amber Dahlin3, Andrea H Ramirez2, Dan M Roden2, Russell A Wilke2, Cathy A McCarty3, Robert L Davis3, Claire M Brett2, Kathleen M Giacomini1. Dept of Bioengineering and Therapeutic Sciences, Univ of California San Francisco1, San Francisco, CALIFORNIA; Dept of Medicine, Vanderbilt Univ Medical Center2, Nashville, TENNESSEE; Center for Human Genetics, Marshfield Clinical Research Foundation3, WISCONSIN; Kaiser Permanente Georgia, Center for Health Research Southeast4, Atlanta, GEORGIA. Dept of Anesthesiology, Univ of California San Francisco5, San Francisco, CALIFORNIA.

Introduction. Inter-individual variation in response to metformin, first-line therapy for type 2 diabetes, is substantial. Previously, transporters have been shown to contribute to the inter-individual variability in metformin pharmacokinetics and pharmacodynamics.

Aims. We examined the effects of promoter variants in both, MATE1 (g.-66T>C, rs2252281) and MATE2 (g.-130G>A, rs12943590) on variation in metformin disposition and response in ethnically diverse healthy subjects and type 2 diabetic patients.

Methods. The pharmacokinetics and glucose-lowering effects of metformin were assessed in predominantly African-American healthy subjects (n=57) receiving metformin (1850 mg). Using electronic health records, the relative change in HbA1c within 3-9 months following initiation of metformin monotherapy was examined in predominantly Caucasian type2I diabetic patients (n=249). Statistical analysis was conducted using Student’s t-test and regression analysis.

Results. In healthy subjects, the renal and secretory clearances of metformin were higher (22% and 26%, respectively) in carriers of variant MATE2 (-130G>A) who were also MATE1 (-66T>C) reference (n=32, P<0.05). Furthermore, both MATE genotypes were associated with altered post-metformin glucose tolerance, with variant carriers of MATE1 (-66T>C) and MATE2 (-130G>A) having an enhanced (P<0.01) and reduced (P<0.05) response, respectively. Consistent with these results, diabetic patients carrying the MATE1 (-66T>C) variant and OCT1 reference allele showed greater relative reduction in HbA1c (n=145, mean [95%CI], -0.12 [-0.14-0.09]) compared to those carrying the MATE1 reference (-0.16 [-0.20-0.12], P=0.01), after adjustment for dose and ethnicity.

Discussion. These findings suggest that promoter variants of MATE1 and MATE2 are important determinants of metformin disposition and response in healthy subjects and type 2 diabetic patients. Further, the study provides evidence that MATE1, MATE2 and OCT1s work in concert and should be considered together when ascertaining the genetic determinants of renal elimination and response to metformin. Finally, the results of our study suggest an important role of MATE2 in the pharmacokinetics and pharmacodynamics of metformin.

**163**

**Gentamicin directed therapy: Which program to use?**

Shaun S Kumar1,2, Craig Wong1,2, Garry G Graham1,2, Evan E Beggs3,4, Paul KL Chin3, Jonathan Brett2, John E Ray2, Deborah JE Marriott5, Kenneth M Williams1,2, Richard O Day1,2. School of Medical Sciences, Univ of New South Wales1, Kensington, NSW; Dept of Clin Pharmacol, St Vincent’s Hosp2, Dept of Clin Pharmacol, Christchurch Hosp3, Christchurch, NZ; Dept of Med, Univ of Otago4, Christchurch, NZ; Dept of Clin Micro & Infectious Diseases, St Vincent’s Hosp5, Darlinghurst, NSW.

Introduction. Australian Therapeutic Guidelines recommends initiating directed therapy of gentamicin if administration exceeds 48 hours. The directed dose of gentamicin is based on a 24-hour AUC using gentamicin plasma concentrations and is calculated by a dosing prediction program. TCIWorks or Aladdin have been suggested for this purpose but the outputs have not been assessed for concordance.

Aims. To determine if the directed doses predicted by TCIWorks, Aladdin and Excel (adapted from Beggs et al., 1995) agreed with those predicted using Abbottbasse.

Methods. Retrospective peak and trough plasma concentrations after the first and second administered doses of gentamicin were available for three groups (n=20-23) of varying creatinine CLs (<40 mL/min, 40-80 mL/min and >80 mL/min). The directed dose needed to produce 24-hour AUC values of 80 mg.h/L was calculated using each program. The qualification was that the peak concentrations must be >10 mg/L and trough concentrations <0.5 mg/L. If the qualification criteria were not satisfied, the dosage interval was extended to 36 or 48 hours and the AUC target was adjusted proportionally to 120 or 160 mg.h/L, respectively. Agreement of dose predictions was examined by Bland-Altman analysis.

Results. The ratio (95% agreement limits=1.96*SD) of the directed doses determined following the first administered dose of gentamicin by TCIWorks, Aladdin and Excel compared to Abbottbasse were 106% (96% to 116%), 102% (87% to 118%) and 108% (91% to 125%), respectively. Similar mean ratios were seen following the second dose of gentamicin. For each of the three renal function groups, the programs yielded similar directed doses compared to Abbottbasse.

Discussion. The four programs used in the calculation of directed doses of gentamicin yielded similar results. Any would be suitable for use in clinical practice. Prospective comparison of all four packages is still required.

Clozapine-induced myocarditis: characterisation using case-control design
Kathlyn J Ronaldson1, Paul B Fitzgerald2, Andrew J Taylor3, Duncan J Topliss4, Rory Wolfe1, John J McNeil1
1Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria; 2Monash Alfred Psychiatry Research Centre, Monash University and Alfred Hospital, Melbourne, Victoria; 3Heart Centre, Alfred Hospital, Melbourne, Victoria; 4Department of Endocrinology & Diabetes, Alfred Hospital, Melbourne, Victoria.

Introduction. Myocarditis is a hypersensitivity reaction, typically occurring in the third week after commencing clozapine, the most effective treatment available for schizophrenia.

Aims. A case-control design is commonly used to investigate risk factors, we show that it can be used to characterise an adverse reaction more broadly.

Methods. Cases and controls were documented from patients’ medical records. Controls were matched by unit at which clozapine was commenced and approximate start date.

Results. 105 cases and 296 controls met entry criteria. Time to onset for cases was 10-33 days, with 82% developing 14-21 days after commencing clozapine. Almost 90% of cases and controls had tachycardia. Eosinophilia developed in 64% of cases and 30% of controls, but onset among cases was delayed 0-8 days after the peak in troponin. However, 87% of cases had C-reactive protein (CRP) > 50mg/L and CRP could be raised up to 5 days before the rise in troponin.

Multivariate regression analysis indicated that the risk of myocarditis increased with increasing age (31% per decade; 95% CI 7-60%), increasing rate of clozapine dose titration (26% per 250mg during days 1-9; 95% CI 2-55%) and concomitant sodium valproate (odds ratio 2.59; 95% CI 1.51-4.42).

Discussion. Comparison of cases and controls permitted identification of the features of myocarditis, and avoided confounding by features associated with introduction of clozapine. Monitoring for myocarditis should use troponin and CRP but not eosinophil counts. Clozapine should be introduced by slow dose titration and sodium valproate is best avoided, if clinically feasible.

Relationship between high risk prescribing and adverse outcomes in people with and without Alzheimer’s disease
Danijela Gnjidic1,2,3, Sarah N Hilmer2,3, Sirpa Hartikainen4, Anna-Maija Tolppanen4, Heidi Taipale4, Marjaana Koponen5, J Simon Bell1,4,5. Faculty of Pharmacy, Univ of Sydney 1, Sydney, NSW; Clin Pharmocol Dept, Royal North Shore Hosp2, Sydney, NSW; Sydney Medical School, Univ of Sydney 3; Sydney, NSW; Kuopio Research Centre of Geriatric Care, Univ of Eastern Finland4, Kuopio, Finland; Quality Use of Medicines and Pharmacy Research Centre, School of Pharmacy and Medical Sciences, Univ of South Australia5, Adelaide, SA.

Introduction. There is a lack of empirical data in relation to possible negative outcomes associated with use of anticholinergic and sedative medicines in older adults with Alzheimer’s disease (AD). Aims. The objective of this study was to investigate the relationship between exposure to medicines with anticholinergic and sedative properties with hospitalisation and mortality in people with and without AD in Finland. Methods. Community-dwelling individuals (n = 16,897) with AD in 2005 were identified by the Social Insurance Institution. For each person with AD, a comparison person individually matched in terms of age (±1 year), sex, and region of residence was identified. Records of reimbursed medicines purchased from 1st September–31st December 2005 were extracted from the Finnish National Prescription Register. High risk prescribing was defined using the Drug Burden Index (DBI), a dose-normalised measure of exposure to anticholinergic and sedative medicines. Mortality and hospitalisation data were extracted from National Registers. Cox and logistic regression analyses were used to investigate the relationship of high risk prescribing with mortality and hospitalisation over a one-year follow-up period. Results. The age of the participants with and without AD ranged from 65-101 (mean 79.2) years, with women comprising 67% of participants. For every unit increase in DBI, the adjusted hazard ratio (HR) for mortality was 1.22 (95% confidence intervals [CI]: 1.11-1.35) among AD participants, and 1.43 (95%CI: 1.27-1.62) for non-AD participants. In relation to hospitalisation data, for every unit increase in DBI, the odds ratio (OR) for being hospitalised over one year in AD participants was 1.38 (95%CI: 1.31-1.46) compared with an adjusted OR of 1.81 (95%CI: 1.70-1.93) amongst non-AD participants. Discussion. These data imply a dose-response relationship of higher DBI exposure with hospitalisation and mortality in both people with and without AD, with a greater relative risk among individuals without AD.
Risk management plans in the Australian regulatory environment

Natalie Raffoul1, Romano Fois1, Shaun Williams2, Rona Hiam2 & Jane Cook2. Faculty of Pharmacy, Univ of Sydney1, Sydney, NSW; Office of Product Review, Therapeutic Goods Administration 2, Canberra, ACT.

Introduction: A Risk Management Plan (RMP) is a set of pharmacovigilance activities and interventions designed to identify, characterise and minimise risks relating to a medicine. RMPs are commonly required as part of applications submitted to the Therapeutic Goods Administration (TGA).

Aims: To characterise additional pharmacovigilance (APhV) and additional risk minimisation (ARiM) activities and study timeframe compliance as part of RMPs for applications submitted to the TGA.

Methods: A retrospective analysis was conducted for RMPs associated with applications submitted to the TGA between January 2009 to August 2012 for new chemical entities (NCEs), extension of indications (EOIs), major variations (MV; e.g. dose form or route of administration) and other (e.g. new combinations of medicines). APhV activities included planned or ongoing studies at the time of application. ARiM included activities such as Healthcare Professional education.

Results: One hundred and thirty-four (134) applications with RMPs were submitted with approved products, 35% NCEs, 33% EOIs, 17% MVs and 15% other. Approximately 76% of these RMPs proposed at least one APhV activity and 23% ARiM activities. Preliminary results indicate 443 APhV activities were included with RMPs, with 33% planned and 67% ongoing studies. Seventy percent (70%) of planned studies were ongoing or completed at the date of approval. Approximately 70% of studies were ‘on-target’ and within timeframes specified.

Discussion: A large number of APhV and ARiM activities are proposed with applications to the TGA for NCEs, EOIs, MVs and other. Compliance with study timelines within RMPs appears to be good. However, preliminary results indicate most planned studies are already completed or initiated at the date of product approval, suggesting RMPs submitted to the TGA are outdated because they are not reflective of post-marketing pharmacovigilance commitments proposed at the time of submission.
**168**

β-alanine, A GABAc partial agonist becomes antagonist when one residue at loop C of binding pocket mutated

Moawiah M Naffaa, Nathan L Absalom, Mary Chebib, David E Hibbs and Jane R Hanrahan. Faculty of Pharmacy, University of Sydney, Sydney, NSW

Introduction: The GABAc Receptors are member of the LGIC superfamily. They are structurally related to GABA<sub>B</sub> receptors but have a distinct pharmacology (1). Actually, extensive research has been done on GABAc receptors in order to identify amino acids involved in GABA<sub>B</sub>-mediated activation. Recently, the functionality of threonine 244 residues has been studied with various ligands (2). In this study we continue our investigation on T244 with the partial agonist β-alanine.

Aims: Structural and functional studies of residues located at the extracellular domain of GABAc receptor.

Methods: 1. Schrödinger suite 2012, Glide 5.8; was used to study β-alanine docking into the receptor homology model (3) 2. Site-directed mutagenesis; used to prepare mutant receptors. 3. Two Electrode voltage clamp electrophysiology used to measure the response of receptors to β-alanine (4).

Results: β-alanine activity has been tested on GABAc WT and T244S mutant. On WT, β-alanine acts as a partial agonist by activating only 50% of the GABA<sub>B</sub> response (10 μM). On the other hand, when applied to the T244S mutant, a drastic decrease in sensitivity was noticed, and instead it becomes an antagonist (Figure 2).

Discussion: Our docking studies predict that β-alanine forms a direct hydrogen bond with threonine 244 in loop C of the binding pocket (Figure 1). The conversion of β-alanine to an antagonist on the T244S mutant indicates that this residue is essential for channel activation. This suggests that the T244 is involved in the early processes of coupling agonist binding to channel gating.


---

**169**

Analgesic efficacy and mode of action of a small molecule angiotensin type 2 receptor antagonist in a rat model of prostate cancer induced bone pain.

Arjun Muralidharan1,2, Bruce D Wyse1,2 and Maree T Smith1,2. Centre for Integrated Preclinical Drug Development1, and School of Pharmacy2, The University of Queensland, Brisbane, QLD, Australia, 4072.

Introduction: Our laboratory has previously shown that single bolus doses of small molecule angiotensin type 2 receptor (AT<sub>2</sub>R) antagonists, produced dose-dependent analgesia in nerve-injured rats by a mechanism that involves blockade of p38 and p42/p44 mitogen-activated protein kinases (MAPK) activation in lumbar dorsal root ganglia (DRGs) (Smith et al., 2012). Hence, the present study was designed to investigate a role for angiotensin II/AT<sub>2</sub>R signaling in the pathobiology of prostate cancer induced bone pain (PCIBP).

Aims: To investigate the analgesic efficacy and mode of action of a selective AT<sub>2</sub>R antagonist, PD123,319, in an AT3B prostate cancer cell (APCCs)-induced rat model of PCIBP.

Methods: Rats received a unilateral intra-tibial injection of 4x10<sup>4</sup> APCCs or heat-killed cells (sham) (Muralidharan et al., 2012). Dose-response curves were generated for single i.v. bolus doses of PD123,319 and the ~ED<sub>50</sub> dose was estimated using nonlinear regression (GraphPad Prism v5.03). The expression levels of phospho-p38 (pp38) and phospho-p42/p44 (pp42/pp44) MAPK were determined in lumbar DRGs using immunohistochemistry (IHC) and western blot analysis at the time of peak analgesia of i.v. PD123,319 (3mg/kg).

Results: The mean ED<sub>50</sub> doses for PD123,319 to alleviate mechanical allodynia and thermal hyperalgesia in the ipsilateral hindpaws were 0.9 (95% CI: 0.5 to 1.5) and 3.7 (95% CI: 1.7 to 8.0) mg/kg i.v., respectively. Administration of PD123,319 but not vehicle, reduced mean DRG expression levels of pp38 and pp42/pp44 MAPK to match the corresponding levels in sham-controls.

Conclusion: PD123,319 produced dose-dependent analgesia in a rat model of PCIBP by a mechanism suggesting that small molecule AT<sub>2</sub>R antagonists hold promise as novel analgesics for the relief of PCIBP.

Attenuation of Toll-like receptor 4 reduces reward-like behaviours in mice
Jonathan Henry W. Jacobsen & Mark R. Hutchinson
Physiology, School of Medical Sciences, University of Adelaide, Adelaide, SA.

Introduction. Alcohol abuse is a significant social and economic problem. Recent evidence suggests alcohol-induced pro-inflammatory central immune signalling may also be involved in the actions of alcohol. Specifically Toll-like receptor 4 (TLR4), a pattern recognition receptor, has found to be essential for many of the actions of alcohol.

Aims. To determine whether TLR4 is involved in the rewarding properties of alcohol in mice

Methods. The TLR4 signaling pathway was analysed using (+)-naltrexone - a TLR4 antagonist, and genetic TLR4 knockout mice. Reward-like behaviour was assessed using two-bottle choice and conditioned place preference. Control experiments to eliminate confounds of taste were also conducted. Finally, to determine the cell type and which TLR4 signalling pathways were activated in response to alcohol, immunohistochemistry was performed.

Results. TLR4 K.O. mice and (+)-naltrexone treated mice demonstrated a reduced preference for consuming alcohol compared to wild-type and saline treated mice respectively (main effect of genotype p<0.0001, and (+)-naltrexone p<0.0001). However, mice did not differ in saccharin or quinine intake (p>0.05). Pre-treatment with (+)-Naltrexone reduced the time spent in alcohol conditioned chamber compared to saline treated mice in conditioned place preference (two-way ANOVA p=0.0005).

Discussion. Genetic and pharmacological TLR4 blockade reduced alcohol preference when assessed by two-bottle choice and conditioned place preference, which is indicative of a reduced reward. This difference was not due to altered taste. Immunohistochemical analysis suggests glia are critical for this response. Collectively, the results suggest that TLR4 contributes to the generation of alcohol-induced reward, suggesting that blocking this signalling may prove beneficial in treating alcohol-abuse disorders.

1. Crews, F et al. (2011) Brain Behaviour and Immunology 24 (S1) 4 – 12.

Potent anti-inflammatory effects of andrographolide and its major metabolite, andrographolide sulfonate
Mitchel Low, Cheang Khoo, Suresh Govindaraghavan, Gerald Muench.
CompleMed, School of Health and Science, University of Western Sydney, NSW; School of Medicine, University of Western Sydney, Campbelltown, Australia (introduced by Gerald Muench, University of Western Sydney, Campbelltown, Australia)

Introduction. Chronic inflammation is a contributing factor for many ageing-related diseases including Alzheimer’s disease (AD). In order to provide effective, yet safe anti-inflammatory treatments, there is a renewed interest in the search of plant based novel secondary metabolites. Andrographolide, an ent-labdane diterpene from an ayurvedic herb Andrographis paniculata has been traditionally used for the treatment of chronic inflammatory diseases. However, andrographolide exhibits poor bioavailability (< 3%), and is known to rapidly metabolize to a sulfonate with unknown potency, which was investigated in this study.

Methods. Anti-inflammatory activity was determined by nitric oxide production in LPS + IFN activated RAW264.7 macrophages (n=3, in triplicate). Cell viability was measured using the MTT reduction assay (n=3, in triplicate).

Results. Andrographolide and its major metabolite, andrographolide sulfonate both demonstrated strong anti-inflammatory activity with IC\textsubscript{50} values of 12.4 ± 0.6 \textmu M and 14.2 ± 0.3 \textmu M, respectively. Both compounds were much more potent than the NSAIDs aspirin and ibuprofen or paracetamol (IC\textsubscript{50} values > 1 mM). The LC\textsubscript{50} concentrations for andrographolide and andrographolide sulfonate were determined to be 272 ± 20 \textmu M and 489 ± 11 \textmu M, respectively.

Discussion. The nearly equipotent anti-inflammatory activity of andrographolide sulfonate (which exhibits > 20 times higher plasma levels than andrographolide), together with its extended half-life, might account for its purported clinical efficacy.
Hypoxia-inducible factor 1 (HIF-1) prolyl hydroxylase inhibitors have neuroprotective actions in a neonatal rat model of hypoxic-ischemic brain injury.

Nicole M Jones1, Adam A Galle1. Dept Pharmacology, University of New South Wales1, Sydney, NSW.

Introduction. Hypoxia-inducible factor-1 (HIF-1) is the key transcription factor regulating the expression of many hypoxia-responsive genes, including erythropoietin and vascular endothelial growth factor. Under normoxic conditions HIF-1α protein is constantly degraded due to HIF-1 prolyl hydroxylase enzymes (PHDs) which hydroxylate proline residues on HIF-1α causing ubiquitination and proteosomal degradation and consequently, constitutive levels of HIF-1α protein are almost undetectable. Hypoxia and drugs that can inhibit PHD activity can cause accumulation of HIF-1 and increase target gene expression. Previously, we have shown that preconditioning with hypoxia and PHD Inhibitors (cobalt chloride (CoCl2) and desferrioxamine (DFX)) can protect the brain against hypoxic-ischemic (HI) brain injury and this protective effect is largely due to expression of HIF-1 and its target genes.

Aims. Here we have examined the neuroprotective effects of PHD Inhibitors administered after brain injury.

Methods. Sprague-Dawley rat pups (postnatal day 7) were anaesthetised with isoflorane (1-5%, via inhalation in oxygen) and underwent a unilateral common carotid artery ligation and were then exposed to 3 hours of 8% oxygen. A single, subcutaneous injection of drug treatments: DFX (200mg/kg), CoCl2 (60 mg/kg), ethyl-3,4-dihydrobenzoate (EDHB; 200mg/kg) or saline vehicle was performed immediately after the HI procedure. One week post-injury, brains were removed for histological analysis, using cresyl violet and immunohistochemistry for the neuronal marker - neuronal nuclear antigen.

Results. This combined HI procedure results in a significant loss of brain tissue in the ipsilateral hemisphere. Treatment with DFX (n=12), CoCl2 (n=12) and EDHB (n=10) significantly reduced the degree of damage in the ipsilateral hemisphere by 38%, 42% and 37%, respectively, when compared with vehicle treated littermate HI only controls (n=18; p<0.05, ANOVA, Dunnett’s post-hoc test).

Conclusion. Our findings indicate that modulation of HIF-1 and its target gene expression after HI brain injury is an effective neuroprotective strategy.
Exploring cannabinoid receptor activity of synthetic compounds identified in “Spice”-related products using novel assays for CB1 and CB2 receptor activation.

Jordyn Stuart¹, Samuel D. Banister²³, Shane M. Wilkinson²³, Michelle Glass⁴, Michael Kassiou²³, Mark Connor¹. Australian School of Advanced Medicine ¹, Macquarie University, Sydney, NSW, School of Chemistry² and Brain and Mind Research Institute ³, The University of Sydney, Sydney, NSW, ⁴Dept Pharmacology, University of Auckland, Auckland, New Zealand.

Introduction. Over the counter/over the internet products that mimic the effects of cannabis are a potentially dangerous alternative to smoking cannabis. These cannabimimetics are structurally unrelated to the components of cannabis and their pharmacological activity is largely undefined.

Aims. Identify if proinflammatory central immune signaling is a novel contributor to opioid reward: Toll-like receptor 4 (TLR4), and its MyD88-dependent signaling.

Methods. Conditioned place preference and hotplate latencies for oxycodone was examined in wild-type Balb/c, TLR4⁻/⁻ and MyD88⁻/⁻ mice.

Results. Oxycodone (20 mg/kg) induced a 127 +/- 31 sec change in preference for the oxycodone-paired environment, compared to 32 +/- 32 sec for TLR4⁻/⁻ and -25 +/- 32 for MyD88⁻/⁻ mice. Two-way ANOVA revealed significant effects of oxycodone (p = 0.049), strain (p = 0.046) and interaction (p = 0.01). Hotplate latency revealed a 5-fold leftward shift in the TLR4⁻/⁻ oxycodone dose response curve (ED50 wild-type 1.36 mg/kg versus TLR4⁻/⁻ 0.26 mg/kg; P < 0.0001) compared to wild-type mice.

Discussion. Collectively, these data indicate that the actions of opioids at classical opioid receptors, together with actions at TLR4/MD2, possibly affects the mesolimbic dopamine system and may explain altered opioid reward behaviors. Thus, the discovery of TLR4/MD2 recognition of opioids as foreign xenobiotic substances adds to the existing hypothesized opioid reward mechanisms, identifies a new drug target in TLR4/MD2 for the treatment of addiction, and provides further evidence supporting a role for central inflammation in drug reward.

Support: ARC DP110100297
**The role of the secretory pathway calcium ATPases (SPCAs) in breast cancer**
Jane M Lee¹, Diana G F Ross¹, Sarah J Roberts-Thomson¹ & Gregory R Monteith¹. School of Pharmacy, The Univ of Queensland¹, Brisbane, QLD.

Introduction. The secretory pathway Ca^{2+} ATPase (SPCA) is a Ca^{2+} pump localized to the Golgi. The Golgi is a major site of protein processing and trafficking, which are pathways that are altered in some cancers. Microarray studies show that SPCA1 is overexpressed in some basal-like breast cancers, a molecular subtype of breast cancer that infers a poor prognosis.

Aims. To identify proteins that have altered expression levels due to SPCA1 silencing in MDA-MB-231 basal breast cancer cells.

Methods. MDA-MB-231 cells were seeded into 6-well plates (75 000 cells/well) and treated with Dharmacon siRNA targeted to SPCA1 or non-targeting control siRNA 24 h after plating. Protein was isolated 72 h post siRNA treatment and SPCA1 knockdown was confirmed using real time RT-PCR and immunoblotting. 2D-DIGE and electrospray ionization tandem mass spectrometry was used as a high throughput method to identify proteins sensitive to SPCA1 silencing in MDA-MB-231 breast cancer cells. Results were confirmed using independent immunoblotting with appropriate antibodies.

Results. A total of 215 protein spots were identified to be significantly different between non-targeting and SPCA1 siRNA treatments. Tandem mass spectrometry was used to identify 20 of these spots, and one of these was determined to be heat shock protein 60 (HSP60). Immunoblotting confirmation from 3 independent experiments demonstrated that HSP60 expression was reduced by 81±2% (n=3, P<0.05) upon SPCA1 silencing in MDA-MB-231 cells.

Discussion. These studies show that 2D-DIGE is a suitable method to identify proteins sensitive to SPCA1 inhibition in MDA-MB-231 breast cancer cells. This approach may be an effective method for studying changes in protein expression in other cancer cell types or with silencing of other Ca^{2+} transporters. Further studies are needed to fully characterize the functional consequences of SPCA1-silencing mediated HSP60 downregulation in breast cancer cells.

**Structure-function analysis of allostERIC and bitopic ligand binding at adenosine A1 receptors.**
Anh TN Nguyen¹, Laura Lopez¹, Patrick M Sexton¹, Arthur Christopoulos¹ & Lauren T May¹, Drug Discovery Biology, MIPS, Monash Univ¹, Parkville, VIC

Introduction. The adenosine A₁ receptor (A₁AR) represents a potential therapeutic target for a variety of disorders. A₁AR ligands can interact with the orthosteric site, a topographically distinct allosteric site, or concomitantly bridge both sites via a “bitopic” mechanism (Valant et al., 2012). A₁AR therapeutic applications would benefit immensely from the rational design of more selective and efficacious A₁AR ligands, however this approach requires greater structural knowledge of the A₁AR binding sites.

Aim. To probe the key residues involved in conferring A₁AR allosteric and bitopic ligand affinity, efficacy and allosteric cooperativity.

Methods. Homology modelling of the A₁AR predicted key residues involved in allosteric and bitopic ligand binding. A₁ARs containing alanine substitutions were stably expressed in FlpINCHO cells. Radioligand binding and ERK1/2 phosphorylation assays were used to investigate the influence of receptor mutations on orthosteric (NECA), allosteric (PD81723), and bitopic (VCP746) ligand affinity, efficacy and cooperativity.

Results. The extracellular mutations, T257A, H264A and E172A, significantly enhanced the affinity of VCP746 but decreased or had no effect on NECA affinity (n=4; p<0.05). Transmembrane mutations, V87A, Q92A, N184A, significantly decreased the positive cooperativity between NECA and PD81723 (n=4; p<0.05).

Discussion. T257, H264 and E172 likely form a hydrogen bond network between extracellular loops 2 and 3. Breaking this network may open up an extracellular cavity to facilitate VCP746 binding (see figure). Residues involved in conferring allosteric cooperativity cluster around a region proximal to the orthosteric site. Structural knowledge gained from these studies will inform ongoing structure-activity studies and rational drug design efforts at this therapeutically relevant receptor family.

178

Glucocorticoids inhibit breast tumour cell migration but increase metastasis to the lung in a mouse model of breast cancer

Ebony R Fietz¹, Shenna Langenbach¹, Nuha Al-Zaubai¹, Cameron N Johnstone², Alastair G. Stewart¹ Department of Pharmacology, University of Melbourne¹, VIC Peter MacCallum Cancer Centre², East Melbourne, VIC

Introduction: Chemotherapy-induced nausea and emesis is commonly treated by the administration of glucocorticoids. Glucocorticoids are also known to influence tumour cell behaviour. We have previously identified a class effect of glucocorticoids in inhibiting serum-induced migration in the human breast tumour cell line, MDA-MB-231, in a 2-dimensional scrape wound healing assay and a 3-dimensional modified Boyden chamber assay. This glucocorticoid effect appears to be dependent on transactivation rather than transrepression. Interestingly, the murine breast tumour cell line, 4T1.2, showed dex-induced inhibition of migration in the 3-dimensional but not 2-dimensional migration assays.

Aims: To investigate the effect of glucocorticoids in a mouse model of breast cancer.

Methods: mCherry-expressing 4T1.2 murine breast tumour cells (500,000) were injected into the 4th mammary fat pad of Balb-c mice. Dexamethasone (dex) was administered sc at 0.1mg/kg/day, commencing 2 days after the tumour was first palpable. Primary tumour and organs were harvested after a further 23 days. DNA was extracted from lung, spine and femur using phenol-chloroform and levels of mCherry were measured using qPCR along with vimentin as a control. mCherry content was assessed as a measure of metastasis.

Results: Dex treatment reduced final body weight (Vehicle: 19±0.3g, Dex: 18±0.3g, P<0.05) but there was no affect on primary tumour weight. There was a significant increase in mCherry content (metastasis) in the lungs of dex-treated mice (Vehicle: 1.0±0.3, Dex: 2.0±0.6, P<0.05).

Discussion: Dexamethasone had no effect on primary tumour growth but increased metastasis to the lung. This effect was opposite to expectations based on previous in vitro studies. Our findings suggest Dex may promote tumour spread. Confirmation of these findings in xenograft models of human breast tumours in mice would lead us to advocate for the use of other non-steroidal anti-emetics in treating breast cancer.

179

Oleoyl-L-Glycine and N-Arachidonyl-Glycine Inhibit the Glycine Transporter GlyT2

Robert J. Vandenberg, Jane E. Carland, Robyn E. Mansfield, Renae M. Ryan, Discipline of Pharmacology, Bosch Institute, University of Sydney, Sydney, NSW, Australia.

Introduction: Concentrations of extracellular glycine in the central nervous system are regulated by Na+/Cl--dependent glycine transporters, GlyT1 and GlyT2. Selective inhibitors of GlyT1 have been developed for the treatment of schizophrenia, whilst selective inhibitors of GlyT2 are analgesic in animal models of pain. We have investigated the inhibitory actions of a series of endogenous lipids on GlyT2.

Methods: Human GlyT2 was expressed in Xenopus laevis oocytes and the inhibitory actions of a series of acylcarnitines and arachidonyl-amino acids on glycine transport was measured using electrophysiological analysis. Results: Oleoyl-L-carnitine was the most potent inhibitor of GlyT2 identified with an IC50 of 340 nM, which is 15-fold more potent than N-arachidonyl-glycine. Both Oleoyl-L-carnitine and N-arachidonyl-glycine are non-competitive inhibitors of GlyT2 and show slow onsets of inhibition and slow washouts. The rate of washout can be greatly increased by the inclusion of beta-cyclodextrin in the wash solution, which suggests that these lipid inhibitors may be acting on the GlyT2 via a lipid exposed site on the transporter. Using a series of chimeric GlyT1/2 transporters and point mutant transporters we have identified a leucine residue in extracellular loop 4 of GlyT2 that confers differences in oleoyl-L-carnitine and N-arachidonyl-glycine sensitivity between GlyT2 and GlyT1.

Discussion: Oleoyl-L-carnitine and N-arachidonyl-glycine represent a novel class of lipid-based inhibitors of glycine transport by GlyT2, which have the potential for further development as analgesics.
A bitopic (orthosteric/allosteric) ligand can differentiate monomeric from dimeric forms of a Family A G protein-coupled receptor

J. Robert Lane¹, Prashant Donthamsetti², Jeremy Shonberg¹, Samuel Dentry¹, Lei Shi⁵, Laura Lopez Muñoz¹, Peter J. Scammells¹, Ben Capuano¹, Patrick M. Sexton¹, Jonathan A. Javitch², Arthur Christopoulos¹
Monash Institute of Pharmaceutical Sciences¹, Monash University, Parkville, VIC. Departments of Psychiatry and Pharmacology², Columbia University, New York, United States. Institute for Computational Biomedicine³, Cornell University, New York, United States

Introduction: The dopamine D₂ receptor (D₂R), a prototypical Family A G protein-coupled receptor (GPCR) is an important therapeutic target for the treatment of central nervous system disorders including schizophrenia. SB269652 was identified as the first drug-like allosteric modulator of this receptor.

Aims: To understand the mechanism of action of SB269652 at the D₂R

Methods: Progressively truncated derivatives of SB269652 were synthesized and characterized at the human D₂R using both functional assays and radioligand binding studies. We used a novel functional D₂R complementation system to control of the identity of the individual protomers comprising a dimeric D₂R signalling unit.

Results: We identified a purely orthosteric pharmacophore and a purely allosteric pharmacophore indicating that the parent molecule represents a hitherto-unappreciated bitopic (dual orthosteric/allosteric) ligand. Using the complementation system we show that SB269652 exerts its cooperative effect by binding in a bitopic mode within one protomer but allosterically modulating the other. Mutational impairment of SB269652 binding to one protomer converts the interaction between the bitopic molecule and dopamine into simple competition, thus indicating that the molecule has the ability to differentiate monomers from dimers via switching between allosteric and competitive pharmacology. We used SB269652 to demonstrate the presence of D₂R oligomers in rat striatum.

Discussion: By utilizing a combination of biochemical, cellular and functional complementation assays, medicinal chemistry, analytical pharmacology and molecular modelling, we have identified and validated a chemical probe with a unique mechanism of action, characterized by a “switch” in pharmacology from allosteric to competitive, depending on whether the interaction is occurring at a functional monomeric versus dimeric (or higher order) Family A GPCR.

Delineating determinants of cooperativity, affinity and bias for mGlu5 allosteric modulators

Karen J. Gregory¹,², Elizabeth D. Nguyen¹, Meredith J. Noetzel², Shaun R. Stauffer², Jason T. Manka², Ya Zhou², Mark L. Turlington², Andrew S. Felts², Kyle A. Emmitte², Colleen M. Niswender², Craig W. Lindsley², Jens Meiler²,³,⁴,⁵ and P. Jeffrey Conn². Drug Discov Biol, MIPS, Monash Univ¹, Parkville, VIC. Departments of Psychiatry and Pharmacology², Columbia University, New York, United States. Institute for Computational Biomedicine³, Cornell University, New York, United States

Introduction. Metabotropic glutamate receptor 5 (mGlu5) has emerged as an exciting new therapeutic target; mGlu5 enhancers are desired for treatment of schizophrenia and cognitive disorders, whilst inhibitors are being sought for autism and depression. Traditionally, efforts have sought to competitively mimic or block glutamate activity. An alternative approach is to target distinct allosteric sites; these compounds are termed allosteric modulators. Negative allosteric modulators (NAMs) inhibit, while positive allosteric modulators (PAMs) enhance, the activity of glutamate. mGlu5 allosteric modulator structure-activity relationships are notorious for being either “steep” or “flat” and also for their propensity to show “molecular switches” whereby a PAM is derived from a NAM scaffold or vice versa. In addition, there is evidence for multiple allosteric sites and biased modulation.

Aims. Identify the molecular determinants that govern mGlu5 allosteric modulator affinity and cooperativity.

Methods. Mutations were introduced into mGlu5, guided by a comparative model of mGlu5 based on the β₂-adrenergic receptor crystal structure. All mutations were assessed for perturbation of allosteric modulation of glutamate induction of intracellular calcium mobilization. Where practical, effects on ligand affinity were quantified utilizing the radiolabelled allosteric ligand [³H]methoxyPEPy.

Results. We identified novel mutations within the transmembrane domains that influence allosteric modulator binding and cooperativity, including residues that differentially affect PAMs compared to NAMs. Interestingly, point mutations in TMs 6 and 7 were discovered that engendered “molecular switches” in modulator cooperativity, such that PAMs became NAMs or neutral modulators or NAMs behaved as PAMs.

Discussion. Use of our homology model combined with the systematic mutagenesis performed will provide tools to drive our understanding of how allosteric modulators exert their effects. Ultimately, these studies will aid drug design efforts, to rationally predict pharmacological profiles and minimize undesirable activities.
Nordihydroguaiaretic acid activates human TRPA1
William J Redmond¹, Liuqiong Gu², Peter McIntyre³, Mark Connor¹. Australian School of Advanced Medicine, Macquarie University¹, NSW, Dept. of Pharmacology, University of Melbourne¹, Parkville, VIC; Australian Health Innovations Research Institute, RMIT University³, Bundoora, VIC.

Introduction. Nordihydroguaiaretic acid (NDGA), is an anti-oxidant and broad spectrum lipoxygenase inhibitor that has been used in topical medications for skin conditions and trialled as an anti-cancer drug. While assessing the role of arachidonic acid metabolites in the activation of the human transient receptor potential ankyrin repeat 1 (hTRPA1) channel, we sought to inhibit lipoxygenase activity with NDGA.

Aims. To define the effects of NDGA at hTRPA1.

Methods. HEK 293 cells stably transfected with hTRPA1 or a mutant where cysteine residues Cys619, Cys639 and Cys663 had been changed to alanine (3xCys mutant) were grown in 96 well plates, loaded with a proprietary calcium sensitive dye (Molecular Devices) and intracellular calcium ([Ca]i) levels measured in a Flexstation 3 microplate reader.

Results. At 37°C, NDGA increased [Ca]i in hTRPA1 expressing cells with a pEC50 of 5.4 ± 0.1, and a maximum increase in fluorescence of 402 ± 30% (n=5). Cinnamaldehyde, a prototypic TRPA1 agonist, elevated [Ca]i with a pEC50 of 4.95 ± 0.05, with a maximum increase of 480 ± 20% (n=5). The effects of NDGA were blocked by the TRPA1 inhibitor HC 030031, and strongly reduced in the 3xCys mutant (100 ± 16% increase in [Ca]i at 100 μM NDGA). The effects of cinnamaldehyde were virtually abolished in the 3xCys mutant (42 ± 7% increase at 300 μM cinnamaldehyde). The maximum effect of both NDGA (550 ± 80%) and cinnamaldehyde (690 ± 40%) were increased when the experiments were performed at 24°C.

Discussion. We have shown that NDGA, a compound widely used to inhibit lipoxygenase activity in studies of animal nociception and previously used as a drug in humans, is an agonist at TRPA1, an ion channel associated with nociception and inflammation. The contribution of actions at TRPA1 to the biological effects of NDGA remain to be established.
**Newer drugs in older people: better the devil you know when it comes to medication safety?**
Gregory Peterson¹ UMORE, School of Pharmacy, University of Tasmania¹, Hobart, TASMANIA.

Australian consumers have been recently advised that they should wait at least seven years from the date of release on the market to take any new drug, unless it is a rare ‘breakthrough’ drug.¹ In part, this was based on analyses of new drug entities approved by the US Food and Drug Administration and subsequent post-marketing labelling changes or product withdrawal after the drugs had been used in large numbers of patients.²³ Similar analyses have been performed on drugs approved in Canada.⁴
The rationale for this advice will be discussed and analysed, with particular reference to the marketing and clinical use of newer antithrombotic drugs.


---

**Complex patients, complex models: PBPK made easy?**
Geoff Tucker¹ ², University of Sheffield UK¹, Simcyp Ltd², Sheffield UK

The application of physiologically-based pharmacokinetic (PBPK) modelling is coming of age in drug development and regulation, reflecting significant advances over the past 10 years in the predictability of key pharmacokinetic parameters from human in vitro data and in the availability of dedicated software platforms and associated data bases. With respect to understanding co-variates and variability, the quantitative impact of drug-drug interactions, age, genetics, racial differences, food effects and pharmaceutical formulation have been assessed. In principle, it is also possible to incorporate pathological features in PBPK models to predict PK in specific disease states defined by aetiology and/or severity. These extensions of PBPK modelling, along with the incorporation of the PK of biologicals and moves towards linking PBPK to pharmacodynamic outcome, are clearly of benefit in understanding extremes of risk in different patient populations as part of the process of drug development. Apart from this application, PBPK also has potential use in the health care arena as an educational tool and for the provision of computerised, ‘point of care’ advice on personalised drug dosage. Multi-drug treatment of the complex patient (e.g. an elderly, obese lady with cardiac failure, rheumatoid arthritis, renal impairment, Alzheimer’s disease, and a ‘poor metaboliser’ to boot) is a considerable clinical challenge. One day, when sufficient information is available on the patient, clinicians may be able to link that person to his or her virtual twin within a PBPK-PD model on an iPad to provide safe, effective, individualised dosage, and to avoid undesired drug-drug interactions. If the physician considers this too complex, the friendly clinical pharmacist will be looking over his/her shoulder to provide further guidance. Although there are no simple solutions to this complexity, we should neither fear nor ignore it.
Pharmacokinetic and pharmacodynamic modelling of PR-104: dissecting the “bystander effect” of hypoxia-activated metabolites
Kashyap Patel1,2, Annika Foehrenbacher1, Kevin O Hicks1 and William R Wilson1. Auckland Cancer Society Research Centre, Univ of Auckland1, Auckland, NZ; Centre for Medicine Use & Safety, Monash Univ2, Melbourne, VIC.

Introduction. Hypoxia is a unique feature in solid tumours and is therefore a potentially exploitable therapeutic target. PR-104, currently in clinical trial, is a phosphate ester that is rapidly converted to its alcohol produg, PR-104A. This produg is activated by reduction to hydroxylamine (PR-104H) and amine (PR-104M) metabolites, both in hypoxic cells and independent of hypoxia by aldo-keto reductase (AKR) 1C3. However, it is unknown whether the high single-agent activity of PR-104 in some human xenografts reflects a “bystander effect”, due to metabolite diffusion from hypoxic zones, or metabolism by AKR1C3.

Aims. To understand the contributions of hypoxia-activated bystander killing and oxygen-independent metabolism in the anti-tumour activity of PR-104.

Methods. Initially, a population model was developed to describe the plasma pharmacokinetics (PK) of PR-104 and PR-104A in rodents, dogs and humans. The pharmacodynamics (PD) of PR-104A and PR-104H was then measured in tissue culture studies, and their extravascular transport investigated using an in vitro multicellular layer (MCL) model. A spatially resolved (SR) model was then developed to predict the PK and PD (cell kill) at each position in a three-dimensional tumour microvascular network.

Results. Population PK analysis estimated rapid conversion of PR-104 to PR-104A, with a faster clearance of PR-104A in dogs and humans than in rodents. In SiHa cervical carcinoma cells, the Area under the concentration-time curve (AUC) was identified as the key exposure variable that correlates with clonogenic cell killing. The diffusion coefficient of PR-104H was similar to that for PR-104A, but its lower metabolic stability gave a calculated diffusion half distance of 50 μm in tissue. SR-PK/PD simulations showed that the monotherapy activity of PR-104 occurs via 3 pathways: hypoxia-activated bystander killing, hypoxia-independent activation and circulating metabolites.

Discussion. While the current SR-PK/PD model under-predicted measured activity in SiHa xenografts, an improved model is under development to further our investigation of the relative importance of bystander cell killing.

Metabolic drug activation in rheumatoid arthritis and drug toxicity
Michael D Wiese1, Sansom Institute for Health Research, University of South Australia1, Adelaide, SA

Introduction. Conventional Disease Modifying Anti Rheumatic Drugs (DMARDs) are the cornerstone of treatment for early rheumatoid arthritis. These drugs tend to have multiple mechanisms of action with respect to both efficacy and toxicity, and metabolic activation is often an important step in achieving both therapeutic and toxic effects.

Aims. To provide an overview of the role that metabolic drug activation plays in the toxic effects of sulfasalazine and leflunomide, including identification of factors that may be used to identify patients that are more likely to cease these drugs due to toxicity.

Methods. Patients who were included in the Royal Adelaide Hospital Early Arthritis inception cohort were included in this retrospective analysis. Patients were treated according to a structured treatment algorithm with a ‘treat to target’ approach. Information regarding patients’ demographic, pathological and genetic variables was collated, and the effect of genetic markers on the rate of cessation due to toxicity with sulfasalazine and leflunomide was determined by a Cox proportional Hazard model.

Results. Cessation due to toxicity was more likely amongst users of leflunomide who were CYP2C19 poor metabolisers (i.e. carried one or two CYP2C19 loss-of-function alleles and no gain-of function alleles). Likewise, NAT2 slow metabolisers were more likely to cease sulfasalazine due to toxicity. ABCG2 genotype did not appear to influence cessation of sulfasalazine or leflunomide due to toxicity.

Discussion. Genetic variables in pathways involved in metabolic drug activation may be able to predict individuals who are more likely to cease sulfasalazine and leflunomide due to toxicity, but further work must be conducted so that this information can be used to personalise treatment with conventional DMARDs and improve outcomes for patients with rheumatoid arthritis.
Structure activity studies and therapeutic potential of venom peptides that target acid-sensing ion channels.
Lachlan D Rash. The University of Queensland, Institute for Molecular Bioscience, St Lucia, QLD.

INTRODUCTION: A marked decrease in pH, or acidosis, is often associated with painful pathological conditions such as inflammation, ischemia, trauma, infection, and malignant tumours (Deval et al, 2011). Furthermore, central nervous system acidosis is a key mechanism of neurodegeneration (in stroke and multiple sclerosis) and seizures (in epilepsy). Acid-sensing ion channels (ASICs) are activated by the drops in pH reached during acidosis (~pH 6-6.5). They are the primary neuronal proton sensors in mammals and play central roles in pain perception and in mediating neurodegeneration that follows acidosis. Inhibition of ASICs has been shown to be analgesic in rodents (1a and 3) and humans (1a)(Deval et al, 2011), and neuroprotective in models of stroke (Pignataro et al, 2007) and multiple sclerosis (Vergo et al, 2011) with minimal side effects. Therefore potent and selective ASIC modulators are very attractive as potential broad range and safe therapeutic leads for pain and neurodegeneration. AIM: To give an overview of the therapeutic potential of peptidic ASIC modulators and the work we are doing to understand their binding sites and the molecular basis of their interaction with these sites and their mechanism of action.

METHODS: We used a combination of chemical synthesis and recombinant expression in E. coli, NMR, two-electrode voltage-clamping of Xenopus oocytes and in silico approaches such as restraint based docking using HADDOCK. RESULTS/DISCUSSION: We have successfully determined new structures and the pharmacophores of two ASIC modulating peptides, PcTx1 (ASIC1a) and APETx2 (ASIC3) both of which are in pre-clinical development. Furthermore, we have discovered the most potent blocker of ASIC1a to date and are carrying out work on its structure, mechanism of action and therapeutic potential.


Consequences of human TRPV4 mutations: implications for drug targeting and disease
Peter McIntyre1, Health Innovations Research Institute, RMIT University1, Bundoora, VIC

Introduction. The TRPV4 cation channel, is widely expressed in mammalian tissues and is the subject of intense study to determine if it has therapeutic potential in a range of diseases. TRP ion channels have diverse roles in sensing environmental stimuli either directly (e.g. TRPV1 and TRPA1), or indirectly like Drosophila TRP, which responds to phospholipase C signalling from light-activated rhodopsin. TRPV4 is activated by osmotic swelling, shear stress, temperature and by agonists, but the physiological mechanisms of its activation are not well understood. Recently, we and others identified point mutations of TRPV4 in humans which produce 3 distinct phenotypes in affected individuals affecting nerves, bone or joint development (see Lamandé et al 2011 for details). We may be able to exploit distinct tissue-specific TRPV4 modulation for new therapies.

Aims. Test activation of TRPV4 by activating endogenous GPCRs or by applying hypotonic solutions in HEK293 cells. Use signalling inhibitors and activators on wildtype and mutant TRPV4 constructs to investigate signalling mechanisms involved.

Methods. TRPV4 activation was assayed by measuring intracellular calcium levels with FURA2 Fluorescence in HEK 293 cells.

Results. Human mutations in the N-terminus reduced TRPV4 responsiveness to hypotonic solutions whereas other human mutations reportedly increase TRPV4 responsiveness. We identified a common signalling pathway for hypotonicity and GPCR activation of TRPV4 and have partially characterised it.

Discussion. TRPV4 mutations have tissue-specific effects suggesting that cell-specific interactions modulate it. TRPV4 appears to be a receptor-operated channel that is activated by intracellular signalling resulting from cell swelling or activation of specific GPCRs. Targeting tissue-specific activation pathways of TRPV4 may be a fruitful area for therapeutics with reduced side-effect potential.

TRPM7 in cardiac hypertrophy: more than just a cation channel
Tamara M Paravicini. School of Biomedical Sciences, The University of Queensland, St Lucia, QLD

Introduction. The transient receptor potential melastatin 7 (TRPM7) channel is a ubiquitously expressed cation channel that is permeable to Mg^{2+} and Ca^{2+}. Unusually for a cation channel, TRPM7 also contains a kinase domain, making this a dual function protein that can both transport cations and activate intracellular signalling cascades. TRPM7 is essential for cell growth and embryonic development, however its role in cardiac cells remains poorly understood.

Aims. This study aimed to determine whether TRPM7 could mediate growth and remodelling of cardiac cells.

Methods. Ventricular cardiomyocytes were isolated from neonatal rats. Cells were then treated with short hairpin RNAs to downregulate TRPM7 expression, or with a recently described pharmacological inhibitor of TRPM7 (NS8593, 10 μM) before being exposed to either angiotensin II (AngII) or neuregulin (NRG) to induce hypertrophy. Hypertrophic signalling was measured using promoter-driven reporter assays for classical ‘pro-hypertrophic’ genes, and reorganization of the actin cytoskeleton examined with phalloidin staining.

Results. Both AngII and NRG caused activation of myosin light chain 2v, atrial natriuretic peptide and cyclin D (indicating an increase in hypertrophic signalling). Downregulation of TRPM7 using three independent shRNAs significantly inhibited the AngII-induced activation of these hypertrophic reporters. Activation of hypertrophic signalling was also reduced by pharmacological inhibition of TRPM7 with NS8593. AngII and NRG both cause sarcomeric re-organization and cellular hypertrophy in cardiomyocytes; this could be prevented by either pharmacological inhibition or downregulation of TRPM7.

Discussion. The TRPM7 cation channel contributes to hypertrophic signalling, cellular growth and reorganization in cardiomyocytes. Further investigations are required to determine whether this is due to Mg^{2+}/Ca^{2+} transport, or activity of the TRPM7 kinase domain.
Systematic Reviews of Adverse Effects – Why Bother?
Yoon K Loke, Senior Lecturer in Clinical Pharmacology, Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, United Kingdom.

While systematic reviews and meta-analyses are top of the evidence hierarchy, most of the work has concentrated on evaluation of treatment benefit. This talks aims to present a structured framework and provide practical relevant examples that illustrate how adverse effects can be evaluated in a comprehensive, unbiased manner. There are unique methodological challenges stemming from the diversity of adverse outcomes ranging from common, mild symptoms to rare, fatal events, thus making it almost impossible to design a single study that addresses all facets. Retrieval of the most appropriate studies should usually be specifically tailored to fit the nature of the adverse effects, according to the primary objective and study question. This depends on whether the main aim is towards scoping/hypothesis-generation, or to statistically calculate magnitude of risk (with hypothesis testing), or clarifying characteristics and risk factors of the adverse effect.

Selection of appropriate data sources depends on characteristics of the adverse effect (e.g. background incidence and effect size of the drug, pharmacological predictability, clinical presentation, time of onset after drug exposure). Reviewers should bear in mind possibility of Type II errors (a particular problem when evaluating rare adverse effects) that lull us into a false sense of security (e.g. wrongly concluding that there was no significant difference in harm between drug and control, with the drug erroneously judged as safe). Hence, it is important to retrieve particular study designs that are most likely to yield robust data on the adverse effects of interest, rather than rely on studies that are poor at measuring certain types of adverse effects, thus leading to ‘false negatives’. Reviewers and readers should also be aware of methodological limitations or controversies in the conduct of meta-analyses that can lead to conflicting or differing interpretations of the dataset.

Public Health: drug safety epidemiology
Dr Kathlyn Ronaldson, Department of Epidemiology and Preventive Medicine, Monash University, VIC

The findings that rofecoxib and rosiglitazone increased the risk of myocardial infarction drew attention to the limitations of spontaneous adverse reaction reporting programs which have been the mainstay of pharmacovigilance since they were established following the thalidomide disaster. Regulators have now moved to a far greater emphasis on active investigation and less emphasis on the passive surveillance of spontaneous reporting. This change includes a requirement that companies submitting an application for a new chemical entity provide a Pharmacovigilance Plan which describes how the company will investigate gaps in knowledge about the safety of their product. Spontaneous reporting programs are not able to determine that a drug increases the risk of an event which has a significant background incidence in the population of individuals taking the drug, and which has no specific features which lead to an association with the drug being made. They also are poor at identifying events with a long time to onset, such as malignancies. Thus the association between rofecoxib and myocardial infarction was made in a randomised controlled trial (RCT), and meta-analysis of RCTs was required for the association with rosiglitazone. Typically adverse reactions, at least the serious ones, are rare, and even meta-analysis may not provide a sufficiently sizable cohort to make an association. Case-control designs are the epidemiological approach to investigating a rare occurrence in the context of a defined exposure. This methodology can be used to investigate associations and also risk factors. Our group has used cases reported to the spontaneous reporting program to investigate risk factors for a number of adverse reactions, including hepatitis with flucloxacillin, cystitis with tiaprofenic acid and myocarditis with clozapine. For the last of these three we plan to conduct a pharmacogenetic analysis shortly.
**Pharmacogenomics and drug safety**

Elizabeth J. Phillips, MD, FRCP, FRACP¹,²,³ Institute for Immunology & Infectious Diseases, Murdoch University¹, Sir Charles Gairdner Hospital², Royal Perth Hospital³.

The last decade has seen elucidation of many genes related to drug metabolism, drug transporters and pharmacodynamic factors of specific drugs that have the propensity to drive dose-dependent and pharmacologically predictable adverse drug reactions and drug interactions. Associations between immune response genes such those in the major histocompatibility complex (HLA) have shed light on the immunopathogenesis of serious adverse drug reactions such as drug hypersensitivity syndromes (DRESS/DIHS), Stevens-Johnson Syndrome and toxic epidermal necrolysis (SJS/TEN). The association between HLA-B*5701 and abacavir has been a notable discovery which has acted as a roadmap for T1 → T4 translation from discovery of an association between a gene and a drug toxicity through to clinical and laboratory translation, through to implementation of a screening test which has now been routinely used as part of guideline-based HIV clinical practice in the developed world to successfully essentially eliminate abacavir hypersensitivity. Whether a test will translate is driven by characteristics of the drug and the availability of therapeutic alternatives, attributes of the test itself, nature of the drug toxicity, having an environment or individual to champion the test, the ability to generate high levels of evidence to support the clinical utility and cost-effectiveness of the test, the development of appropriate laboratory support, infrastructure and quality assurance, and the design and implementation of appropriate clinical systems. More recent research has provided important structural and functional insights as to how drugs like abacavir and carbamazepine may specifically interact with HLA-B*5701 and HLA-B*1502 respectively to cause hypersensitivity and SJS/TEN. It is now feasible that these approaches examining how drugs specifically interact with HLA and other genes could be applied as part of a pre-clinical pharmacogenomic screening strategy to inform the design and development of safer drugs.

**The safety of medicines in older people**

David G Le Couteur¹, Danijela Gnjidic²,³, Sarah Hilmer³, Centre for Education and Research on Ageing, Univ of Sydney¹; Department of Pharmacy, Univ of Sydney², Kolling Research Institute, Univ of Sydney³, Sydney, NSW

Older people are the major users of medicines. In Australia older adults take between 5-12 different prescription medicines every day and this tends to be higher in frail elderly people and those in residential care facilities. Such use of high medicine is unquestionably associated with increased risk of harms. Our prospective study of 1705 community-dwelling older men (CHAMP study) has shown that high risk prescribing such as polypharmacy, hyperpolypharmacy and a high Drug Burden Index is associated with a variety of poorer outcomes including increased risk of frailty, falls, death and institutionalization, taking into account comorbidities and other subject characteristics. The use of psychotropic medicines including antipsychotics and opioid analgesics is very prevalent in older people and the use is increasing dramatically in these age groups particularly those in residential care. Yet these medicines have been linked with significant adverse effects including an increased risk of death in older people, especially those with dementia. Such prescribing is usually undertaken in the absence of good quality evidence for the efficacy or effectiveness of these medicines in very elderly people or those with comorbidities. On the other hand, deprescribing and reduction of drug burden has been linked with beneficial outcomes or at least the absence of harm in many situations. This critical situation will only be resolved by including real life frail older people in clinical trials or at least high quality observational studies. Moreover, research is required to establish the benefits of deprescribing in older people with polypharmacy and to develop educational methods that are effective at improving the prescription to older people.
Utilizing oligoarginine to enhance cellular uptake of PECA nanoparticles
Jasper Z S Chiu, Ian G Tucker, Bernie J McLeod, Arlene McDowell. School of Pharmacy, University of Otago, Dunedin, New Zealand.

Introduction. Cell-penetrating peptides, such as oligoarginine, can be covalently bonded with bioactives to enhance their cellular uptake (Fonseca et al, 2009). However, this modifies the bioactive moiety and so potentially its bioactivity. An alternative approach is to associate arginine with nanoparticles that encapsulate the bioactive, allowing the bioactive to be delivered in its native form.

Aims. To compare covalent and non-covalent association of oligoarginine with nanoparticles for improving cellular uptake.

Methods. Poly(ethyl-cyanoacrylate) (PECA) nanoparticles were prepared using a microemulsion template. Oligoarginine of different chain lengths attached to histidine (RRH or R4-aca-H) and FITC-dextran (MW = 2,000 kDa) were added to the aqueous phase of the microemulsion prior to polymerization. Covalent binding of the oligoarginine via histidine anchoring to the histidine residue was determined using MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization – Time Of Flight) spectrometry. Flow cytometry was used to quantify the cellular uptake of the different nanoparticle formulations into Caco-2 cells.

Results. Di-arginine-histidine (RRH) covalently bound to PECA nanoparticles and had a higher uptake than the unmodified PECA nanoparticles. R4-aca-H did not covalently bind to the PECA nanoparticles, however when it was encapsulated, 80% cell uptake was observed.

Discussion. Associating oligoarginine either covalently or entrapping it within PECA nanoparticles increases cellular uptake compared with nanoparticles without arginine. Encapsulated oligoarginine resulted in greater uptake compared to covalently-tagged PECA nanoparticles. Polymeric nanoparticles administered with cell-penetrating peptides may thus have potential to improve absorption of bioactives.

**SPEAKER ABSTRACTS**

**Enhanced exposure of a chemotherapeutic agent to the lymphatic system with the use of nano-sized and PEG-capped drug vectors**

Gemma M. Ryan¹, Lisa M. Kaminskas¹, Michelle P. McIntosh¹, Brian D. Kelly², David J. Owen² and Christopher J.H. Porter¹. Monash Institute of Pharm Sci, Monash Univ ¹, Parkville, VIC; Department of Pharmaceutical Chemistry, University of Kansas², Lawrence, KS, USA.

**Introduction.** The lymphatic system plays an important role in immune function, as well as serving as the main conduit for transport of dietary triglyceride (in the form of lipoproteins) from the intestine. The current study examines the use of triglyceride(TG)-mimetic prodrugs to target the delivery of an immunomodulatory drug, mycophenolic acid (MPA), to the lymphatic system via integration into triglyceride metabolic pathways. Enhanced immunomodulator exposure to sites of action within lymphoid tissue is expected to improve the treatment of immune system diseases.

**Methods.** Biotransformation of the prodrugs was assessed via incubation with rat digestive fluid and rat plasma, and analysis of prodrug derivatives in mesenteric lymph was facilitated by HPLC-MS. Lymphatic drug transport was examined in mesenteric lymph-cannulated rats following intraduodenal drug/prodrug administration under conditions modulated by metabolic enzyme inhibitors.

**Results.** All four TG-mimetics markedly increased lymphatic drug transport (2-96 fold) when compared to parent MPA where lymphatic transport was low (0.14% of dose). Prodrugs were digested rapidly (<2min) by rat digestive fluid to form the monoglyceride equivalent prodrugs (MEPs) prior to being re-esterified with fatty acids in enterocytes. Lipolysis was a prerequisite for efficient lymphatic transport as inhibition of MEP generation by orlistat (320μM) markedly attenuated lymphatic recovery (MPA-TG, 13.5% vs 1.3%, in the absence and presence of orlistat). Stabilisation of labile MEPs by steric hinderance of the ester bond showed both improved (MPA-C6-Me-es-TG, 13.5%) or reduced (MPA-C6-et-TG, 0.31%) lymphatic transport when compared to MPA-C6-es-TG (9.6%), depending on the impact of structural modification on re-esterification.

**Discussion.** The current mechanistic evaluation of the drivers of lymphatic transport of TG-based prodrugs revealed the need for luminal liberation of MEPs, the prevention of further breakdown of MEPs to parent drug (MPA) and derivatives, and the need for subsequent re-esterification to the TG forms in the enterocyte.

---

**Bio-mimetic prodrugs to promote lymphatic transport of immunomodulators: A balance between digestive stability and ease of re-esterification**

Sifei Han¹, Luojuan Hu¹, Tim Quach¹, William N Charman¹, Valentino J Stella², Natalie L Trevaskis¹, Jamie S Simpson¹ and Christopher JH Porter¹. Monash Institute of Pharmaceutical Sciences, Monash University¹, Parkville, VIC; Department of Pharmaceutical Chemistry, University of Kansas², Lawrence, KS, USA.

**Introduction.** The lymphatic system plays an important role in immune function, as well as serving as the main conduit for transport of dietary triglyceride (in the form of lipoproteins) from the intestine. The current study examines the use of triglyceride(TG)-mimetic prodrugs to target the delivery of an immunomodulatory drug, mycophenolic acid (MPA), to the lymphatic system via integration into triglyceride metabolic pathways. Enhanced immunomodulator exposure to sites of action within lymphoid tissue is expected to improve the treatment of immune system diseases.

**Methods.** Biotransformation of the prodrugs was assessed via incubation with rat digestive fluid and rat plasma, and analysis of prodrug derivatives in mesenteric lymph was facilitated by HPLC-MS. Lymphatic drug transport was examined in mesenteric lymph-cannulated rats following intraduodenal drug/prodrug administration under conditions modulated by metabolic enzyme inhibitors.

**Results.** All four TG-mimetics markedly increased lymphatic drug transport (2-96 fold) when compared to parent MPA where lymphatic transport was low (0.14% of dose). Prodrugs were digested rapidly (<2min) by rat digestive fluid to form the monoglyceride equivalent prodrugs (MEPs) prior to being re-esterified with fatty acids in enterocytes. Lipolysis was a prerequisite for efficient lymphatic transport as inhibition of MEP generation by orlistat (320μM) markedly attenuated lymphatic recovery (MPA-TG, 13.5% vs 1.3%, in the absence and presence of orlistat). Stabilisation of labile MEPs by steric hinderance of the ester bond showed both improved (MPA-C6-Me-es-TG, 13.5%) or reduced (MPA-C6-et-TG, 0.31%) lymphatic transport when compared to MPA-C6-es-TG (9.6%), depending on the impact of structural modification on re-esterification.

**Discussion.** The current mechanistic evaluation of the drivers of lymphatic transport of TG-based prodrugs revealed the need for luminal liberation of MEPs, the prevention of further breakdown of MEPs to parent drug (MPA) and derivatives, and the need for subsequent re-esterification to the TG forms in the enterocyte.
Expression, regulation, deorphanisation and putative nutrient-sensing function of taste GPCRs in the heart
Simon R Foster¹, Enzo R Porrello¹, Brooke Purdue¹, Peter Molenaar², Eugeni Roura³, Wolfgang Meyerhof⁴ and Walter G Thomas¹. Sch of Biomedical Sciences¹, Sch of Medicine², Centre for Nutrition & Food Sciences³, Univ of Queenslan, Brisbane, QLD; Dept of Molecular Genetics, German Institute of Human Nutrition (DIfE) Potsdam-Rehbruecke⁴, Nuthetal, Germany.

Introduction. G protein-coupled receptors (GPCRs) are critical for cardiovascular physiology, yet only a small fraction of the cardiac-GPCR repertoire is therapeutically targeted. Taste receptors are functional beyond the mouth, but have not been studied in heart.

Aims. To investigate taste GPCR expression, regulation and function in human and rodent heart.

Methods. RT-qPCR taste receptor screens were performed on failing human hearts, in rodent heart, and in cultured cardiac myocytes and fibroblasts. Taste GPCR localisation was investigated using in situ hybridisation and Tas1r1 gene-targeted mice (Tas1r1CvR/Rosa26RFP). Five cardiac-expressed type 2 taste receptors (Tas2) GPCRs were cloned from rat heart and screened against 102 natural or synthetic bitter compounds in a heterologous expression system.

Results. Subsets of TAS1/Tas1 and TAS2/Tas2 family GPCRs were identified in human and rodent hearts, were enriched in cultured rat myocytes, and were localised in distinct myocardial cells. Following starvation, several Tas2s were upregulated ~3-fold in cultured rat myocytes and in the mouse heart in vivo. We identified novel agonist ligands for three Tas2 GPCRs (Tas2r108, Tas2r137 and Tas2r143). Ongoing work is focused on testing the endogenous cardiac-expressed taste GPCR responses in primary rat ventricular myocytes.

Discussion. The discovery of taste GPCRs in the heart opens an exciting new field of cardiac research. We predict that these taste receptors may function as cardiac nutrient sensors and using our identified agonist ligands, we hope to delineate the physiology of taste receptor activation in the heart.

A reduction in random between subject variability is not mandatory when adding a new covariate
Chakradhar V Lagishetty¹, Pavan Vajjah², Stephen B Duffull¹
School of Pharmacy, University of Otago¹, Dunedin, NewZealand; Simcyp Ltd², Sheffield, United Kingdom

Introduction. Population PKPD analyses include models for heterogeneity between subjects. The remaining between subject variability that cannot be explained by patients’ characteristics, known as covariates, is assumed to arise from random variability. Random variability should decrease when more variability can be explained from predictable sources (e.g. weight or genetics).

Aim. To explore circumstances where random variability may not reduce even though a significant covariate is included in the analysis.

Methods. This work is based on simulation and estimation and does not include any patient data. Simulations were performed using the software MATLAB (ver 2010b) and estimation using the software NONMEM (ver 7.2). For simplicity we assumed the underlying PK was described by an iv bolus 1-compartment model. Five scenarios were considered with correlations between clearance (CL) and the theoretical covariate ranging from 0-100%. We considered two relationships between CL and the covariate, (1) where the relationship had a forced intercept of zero – reflecting when the covariate value (e.g. weight) equals zero then CL equals zero and (2) where we allowed for an intercept, i.e. when the covariate value equals zero (e.g. weight) then CL equals a positive value. Each simulated scenario was also estimated by a base model (i.e. no covariate) which was used for comparisons.

Results. Relationship 1 resulted in inflated random variability with correlations 0 - 75%. Models that allowed for an intercept performed well with slight inflation which was evident only at low correlation (25%).

Discussion. A moderate to strong covariate may not reduce random variability and in fact it may inflate this variability when not correctly implemented. We note here that correct implementation should not necessarily be based on what appears to be a biologically plausible relationship. This would lead to wrong conclusion that covariate was not relevant.
Calcium dependent regulation of multidrug resistance-associated protein 3 (MRP3/ABCC3) gene expression in a model of epithelial-mesenchymal transition (EMT)

Teneale A. Stewart, Iman Azimi, Felicity M Davis, Sarah J. Roberts-Thomson & Gregory R Monteith. School of Pharmacy, The University of Queensland, Brisbane, QLD.

Introduction. Increased expression of members of the ATP binding cassette (ABC) transporter superfamily in cancer cells is linked to chemoresistance, and more recently has been correlated with malignant progression and a more aggressive cancer phenotype. Epithelial-mesenchymal transition (EMT) is a process implicated in cancer metastasis and is a feature of an aggressive tumour subtype. Aberrant Ca²⁺ signaling is a feature of tumourigenesis and tumour progression in some cancer types, and has recently been linked to EMT. The relationship between EMT, ABC transporter expression and Ca²⁺ signaling has not yet been fully assessed.

Aims. To quantify specific MRP/ABCC transporter mRNA expression in a model of EMT using the MDA-MB-468 basal-like breast cancer cell line. 2. To assess the effect of intracellular free Ca²⁺ chelation prior to induction of EMT on MRP/ABCC mRNA expression.

Methods. For induction of EMT, MDA-MB-468 cells were treated with epidermal growth factor (EGF, 50 ng/mL) for 24 h. Intracellular free Ca²⁺ chelation was achieved by loading the cells with 100µM BAPTA-AM for 1 h at 37°C prior to treatment with EGF. Quantitative real-time RT-PCR was used to assess changes in MRP1/ABCC1, MRP3/ABCC3 and MRP5/ABCC5 mRNA expression following EGF and/or BAPTA-AM treatment.

Results. EGF-induced EMT in MDA-MB-468 cells resulted in a significant increase in MRP3/ABCC3, but not MRP1/ABCC1 or MRP5/ABCC5, mRNA relative to the control. This effect was abolished in cells pre-treated with BAPTA-AM, indicating that intracellular Ca²⁺ plays an essential role in facilitating the increased expression of MRP3/ABCC3 in MDA-MB-468 cells undergoing EMT.

Discussion. These data reveal a previously unrecognised protective role for ghrelin gene-derived peptides in the cerebral circulation and brain, and highlight their potential as novel approaches for stroke treatment.
Small airway reactivity to constrictors is differentially altered by chronic and acute inflammatory stimuli
Chantal Donovan1, Simon G. Royce2,3, Mimi L.K. Tang2,3, Jane E. Bourke1
Dept of Pharmacology, University of Melbourne1, Parkville, VIC, Dept of Pediatrics, University of Melbourne2, Parkville, VIC, Department of Allergy and Immunology3, Murdoch Children’s Research Institute, Royal Children’s Hospital, Parkville, VIC.

Introduction. The distal lung is an important site of inflammation, remodelling and airway hyperresponsiveness (AHR) in asthma. These changes are evident in mouse models following chronic exposure to ovalbumin (OVA) while lipopolysaccharide (LPS) directly induces airway inflammation. Limited studies have assessed small airway reactivity in vitro following these stimuli.
Aim. To assess small airway reactivity in mouse lung slices following chronic OVA treatment in vivo or acute LPS treatment ex vivo.
Methods. Balb/C mice sensitised with OVA on days 0 and 14, were challenged with nebulized OVA or saline 3 times/week from day 21 to 64, and small airway fibrosis confirmed using Masson’s trichrome stain. Lung slices (150μm) from saline and OVA groups were cultured in the absence or presence of IL-1α (10ng/mL) and/or TNFα (50ng/mL) for 48h. Slices from untreated mice were exposed to LPS (10μg/mL, 48h) and supernatants assayed for TNFα by ELISA. Changes in small airway lumen diameter in response to serotonin (5HT) and methacholine (MCh) were measured using phase-contrast image analysis.
Results. Following OVA challenge, small airway contractile responses to MCh were slower, with a 13-fold loss in potency and reduced maximum relative to saline controls. In vitro incubation with IL-1/TNFα did not alter reactivity in slices from either group. LPS treatment increased TNFα release (175.6 ± 65.9pg/mL, ND in untreated slices, n=5) and increased airway reactivity to 5HT, but not MCh.
Conclusion. Airway hyporesponsiveness following OVA challenge, even in the presence of inflammatory cytokines, was unexpected and may reflect the influence of fibrosis to oppose small airway narrowing. Increased cytokine release and reactivity with LPS provides conditions to assess the clinical potential of novel anti-inflammatory and bronchodilator therapies targeting the small airways.

Characterisation of the hybrid orthosteric/allosteric bitopic nature of TRPB at the M₁ muscarinic ACh receptor
Peter Keov1, Celine Valant1, Shane M. Devine2, J. Robert Lane1, Peter J. Scammells2, Patrick M. Sexton1, Arthur Christopoulos1. Drug Discovery Biology1 and Medicinal Chemistry2, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Melbourne, VICTORIA.

Introduction. Therapeutics targeting the M₁ muscarinic ACh receptor (mAChR) hold promise in the treatment of cognitive dysfunctions (Langmead et al., 2008). Various novel selective agonists (NSAs) have been identified for the M₁ mAChR and are purported to solely engage the receptor via an allosteric site, rather than the classical ACh (orthosteric) binding site. However, this may not be the case for some NSAs (Valant et al., 2009). We hypothesised that the recently identified NSA TBPB \[1-(1'-(2-methylbenzyl)-1',4'-bipiperidin-4-yl)-1H benzo[d][imidazol-2(3H)-one], may interact simultaneously with both the orthosteric binding site and an allosteric site in a bitopic mode of action (Jacobson et al., 2010; Lebois et al., 2010).
Aims. To characterise the bitopic mechanism of action of TBPB at the M₁ mAChR.
Methods. Truncated fragment molecules of TBPB were synthesised and pharmacologically characterised via radioligand binding and cellular signalling assays using cells recombinantly expressing the M₁, M₂, M₃ and M₄ mAChRs, as well as mutant variants of the M₁ subtype.
Results. Two fragment compounds representing opposite ends of TBPB, VCP794 and VCP813, were identified with pharmacological behaviours consistent with that of orthosteric ligands. Functional studies indicated a molecular moiety of TBPB required for agonism (VCP794), whilst radioligand dissociation kinetics studies revealed a structural moiety required for interaction with an allosteric site (VCP813). Subsequent studies in M₁ mutant receptors identified amino acid residues that differentially affect the binding and function of TBPB and its fragments compared to classical orthosteric ligands.
Discussion. These findings provide evidence of a novel bitopic mode of interaction with the M₁ mAChR by TBPB and highlight the potential existence of more bitopic ligands and their consideration in drug discovery programs.

Simultaneous deletion of the α1α-adrenoceptor and P2X1-purinoceptor generates male mice which are infertile
Carl W White, Jennifer L Short, Sabatino Ventura Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC.

Introduction. Research into male contraceptives has focused on disrupting the production of sperm by altering hormone levels. Such approaches require factors such as the reversibility of such treatments and the efficiency of sperm reduction to be met to be truly suitable targets for a male contraceptive. Furthermore loss of normal hormone signaling frequently results in side effects and impairs sexual function. We have generated α1A-adrenoceptor / P2X1-purinoceptor knockout (DAPKO) mice and observed that males are completely infertile. α1A-adrenoceptors and P2X1-purinoceptors are G protein-coupled receptors and ligand-gated ion channels respectively and have numerous physiological functions. In blood vessels and the genitourinary tract these receptors are primarily involved in smooth muscle contraction.

Aims. To investigate the mechanism of infertility caused by dual α1A-adrenoceptor / P2X1-purinoceptor knockout (DAPKO) in male mice.

Methods. Sexual behaviour and breeding was observed by behavioural video analysis. Sperm motility and viability was investigated by microscopy and intracytoplasmic sperm injection (ICSI). Contractile responses to electrical field stimulation and exogenously applied agonists of genitourinary tissues from DAPKO mice was observed using functional isolated organ bath studies.

Results. Crossing of male DAPKO mice with non DAPKO female mice (n=16) did not result in pregnancy despite copulation occurring with normal vigour and libido. Sperm taken from the epididymis of DAPKO mice were motile (n=6) and could readily fertilise oocytes following ICSI (n=3). Testicular weight was not changed in DAPKO mice however the contractile response of the vas deferens was markedly impaired by α1A-adrenoceptor / P2X1-purinoceptor deletion (n=6, p<0.01). Discussion. These data show that infertility observed in male DAPKO mice is due to a loss of contractile function of the mouse vas deferens which prevents the transport of sperm from the epididymis to the urethra. Selective dual pharmacological blockade of these receptors may be a fast acting, safe and effective non-hormonal male contraceptive.
Can software assist the home medicines review process by identifying clinically relevant drug-related problems?

Colin M Curtain1, Ivan K Bindoff1, Juanita L Westbury1, Gregory M Peterson1. School of Pharmacy, University of Tasmania1, Hobart, TAS.

Introduction. Home medicines reviews (HMRs) are conducted by accredited pharmacists to detect and address drug-related problems (DRPs), to improve the quality use of medication. Identification of DRPs involves an assessment of existing drug treatment in conjunction with other factors. Australian decision support software, Medscope Medication Review Mentor (MRM), has been developed to assist the identification and resolution of DRPs.

Aim. This study assessed the ability of MRM to identify clinically relevant DRPs and to provide suitable recommendations for DRP resolution.

Methods. HMR information and pharmacist-identified DRPs and recommendations were obtained from a database of almost 700 Australian HMRs collected for another project. The HMR information was entered into MRM and findings were recorded. A random sample of 20 HMRs with DRPs found by pharmacists (N=73) and MRM (N=125), were independently assessed by a panel of 12 clinical pharmacology and pharmacy experts. Experts were blinded to each source of DRPs and provided Likert scale responses regarding clinical relevance and recommendation appropriateness.

Results. Experts agreed that the pharmacists (645 of 876 opinions; 74%) and MRM (1092 of 1500; 73%) identified clinically relevant DRPs. There was no significant difference between pharmacists and MRM regarding clinical relevance (Wilcoxon Rank Sum Test, W = 674591, p = 0.212). There was a significant difference regarding recommendation appropriateness (W = 568346, p < 0.001) reflecting greater support for MRM’s recommendations.

Discussion. MRM identified more DRPs than pharmacists but not at a cost of irrelevance. The software’s recommendations compared favourably with those of the pharmacists. MRM appears capable of identifying clinically relevant DRPs and providing appropriate recommendations for DRP resolution.

Safe to crush? Pilot study into solid dosage form modification in aged care.

Greg J Kyle, Nicole Mercovich. Discipline of Pharmacy, University of Canberra, Canberra, ACT.

Introduction. Dosage form modification such as tablet crushing is common practice in ACFs for residents who have difficulty swallowing. (Stubbs et al, 2008) Crushing solid dosage forms can alter their efficacy and safety parameters with clinically significant consequences. (Haw et al, 2010)

Aims. To observe solid dosage form modifications in participating ACFs within the ACT, identify commonly modified medications and the methods employed, and determine aged care staff levels of self-perceived medication knowledge, and the types and quality of resources currently available to staff regarding dosage form modification.

Methods. Medication rounds in a convenience sample of ACT ACFs were observed by MN. Nursing staff knowledge of dosage form modification and available resources was assessed by written anonymous questionnaire. Data were collated to assess the range of modifications and knowledge level.

Results. From 160 medication observations, 29 residents had a total of 75 medications modified by nursing staff prior to administration, with 32% of these medications modified inappropriately. The methods used for crushing and administration resulted in drug mixing, spillage, and incomplete dosing. Staff reported adequate resources; however a lack of knowledge on how to locate and use these resources was found.

Discussion. Mixing all medications together is not recommended, but was observed in all cases. Incomplete or no cleaning of equipment between residents was observed and has the potential to cause adverse reactions. No staff were observed to use available resources for doseform modification. These results show medication crushing is common practice in ACF and many of these modifications are inappropriate.

An observational study of pharmacists’ interventions to minimise medication misadventures in paediatric inpatients
Hesty U Ramadaniati1, Jeff D Hughes1, Ya P Lee1. School of Pharmacy, Curtin Univ1, Bentley, WA.

Introduction. It has been widely documented that medication misadventures present a significant burden on the health care system. There has been extensive research to investigate medication misadventures in adults, yet data is still lacking in the paediatric population. The lack of effective strategies to address this safety issue has significant ramification in children despite being a very vulnerable patient group. However, previous studies have claimed that ward-based clinical pharmacists’ intervention plays a role to reduce the incidence of medication errors in hospital.

Aims. This study aimed to document and evaluate the role of pharmacists’ interventions in minimising medication misadventures in paediatric inpatients at a paediatric hospital in Perth.

Methods. Clinical interventions performed by ward-based clinical pharmacists in two general medical wards during 35-day data collection period were observed and documented by the primary investigator. The pharmacists’ intervention data were analysed to identify the occurrence of medication misadventures.

Results. It has been revealed that a total of 298 interventions were performed for 1215 patients. The rates of intervention were 5.2/100 medication orders and 24.5/100 patients. Approximately 9 interventions were performed per day. Taking medication history/counselling activities were the most common interventions (66.1%), followed by provision of drug information (11.1%) and drug therapy changes (10.4%). When categorised according to therapeutic groups, antiinfectives and analgesics accounted for the top two medications implicated in the interventions. Of all interventions, 44 interventions (14.8%) were considered active interventions. Nearly all pharmacists’ active interventions identified medication errors (n=42) and the majority of errors were intercepted by pharmacists.

Discussion. Pharmacists through their interventions contributed substantially to patient safety in paediatric area by minimising the occurrence of medication misadventures in the study population.

Prevalence and factors associated with antidepressant use in Tasmanian nursing homes
Laura Trillini1, Juanita Westbury1, Gregory Peterson1. Unit for Medication Outcomes1, Research and Education, School of Pharmacy, University of Tasmania, Hobart, TASMANIA.

Introduction. The rate of depression in nursing home residents is high. As depression is associated with increased morbidity and mortality, there has been an increased focus on effective treatment in this setting.

Aims. The aim of this study was to examine the prevalence and pattern of antidepressant use in nursing home residents. Analysis of commonly used antidepressants, dosages and concurrent use of other psychotropic drugs will enhance understanding of depression treatment choices and identify predisposing factors associated with the use of antidepressants in this setting.

Methods. This retrospective cross-sectional study involved the analysis of 562 de-identified Residential Medication Management Review (RMMR) case notes collected by accredited pharmacists in seven nursing homes in Hobart, Tasmania. Residents’ demographics, prescribed medications and medical diagnosis details were extracted. Details on antidepressant use were recorded. Bivariate analysis examined factors associated with antidepressant prescribing. Multivariate logistic regression analysis was used to examine independent predisposing factors associated with antidepressant prescribing.

Results. The overall prevalence of antidepressant use in the nursing homes was 46%. Mean residents’ age was 85.1 years, 72.8% were females. SSRIs were the most commonly prescribed class of antidepressants (20.8%), followed by SNRIs (6.9%) and TCAs (6.9%). Mirtazapine was the most commonly prescribed individual antidepressant (13%). Antidepressant prescribing was associated with concurrent benzodiazepine use and age, with the odds of receiving an antidepressant lower in residents aged > 85 years. Documented history of depression increased the likelihood of antidepressant prescribing, as did a history of chronic pain and anxiety. A history of falls/fractures was not found to be associated with the antidepressant use.

Discussion. Prevalence of antidepressant prescribing in nursing homes is high at 46%. Predisposing factors included younger age, depression diagnosis, presence of chronic pain/anxiety and concurrent benzodiazepine use. Further studies are needed to examine appropriateness of antidepressant use in this demographic.
Pharmacy staff perspectives on patient safety issues involved in the supply of dose administration aids
Ramesh L Walpola¹, Timothy F Chen¹, Andrew J McLachlan¹², Romano A Fois¹. Faculty of Pharmacy, The University of Sydney¹; Centre for Education and Research on A geing, Concord Repatriation General Hospital², Concord, NSW

Introduction: Dose Administration Aids (DAAs) are used to assist patients and health facilities manage medication and reduce administration errors. However, their preparation and use is not without risk. DAA quality assurance research has highlighted stability issues and identified types and rates of error that occur but has not explored broader systems and social issues affecting product quality and patient safety.

Aims: To conduct a qualitative exploration of pharmacy DAA dispensing processes and identify factors that impact quality and compromise patient safety.

Methods: Community pharmacy staff were purposively recruited from metropolitan Sydney to participate in focus groups, discussing processes in DAA service provision, associated problems and possible solutions. Transcripts were analysed to identify recurring themes.

Results: Despite variability in supply processes among pharmacies, focus groups identified a number of common, often interacting, factors that can underlie risk to patient safety; to which participants suggested some solutions. These factors align with categories identified by Phipps et al., 2009, including: inter-professional and patient relationships; demands on the pharmacist; workplace design and management; procedural standards and resource issues. Common elements that can affect safe practise included effective communication, workplace culture and the value pharmacy staff and managers attribute to DAA services.

Discussion: Participants suggested potential solutions to mitigate error including formalised training and plain-English guidelines; however, effective solutions need also address broader cultural, social and economic drivers of quality.


Prevalence of frailty and number of medicines use in elderly Australian: A comparison of four frailty measures
Imaina Widagdo¹, Nicole Pratt¹, Mary Russell², Libby Roughead¹. School of Pharmacy and Medical Sciences, University of South Australia¹, Adelaide, South Australia; School of Health Sciences, University of South Australia², Adelaide, South Australia.

Introduction: Frailty has been associated with an increased risk of adverse health outcomes; however, frailty has not been clearly defined. Several measures to identify frailty have been developed, each using different assessment criteria. Consequently, classification of patients’ frailty status may vary depending on the measure used.

Aims: To compare the prevalence of frailty using four different frailty measures and to examine the number of medicines used among the frail elderly.

Methods: This study used data from the Australian Longitudinal Study of Ageing (ALSA), which is a population based cohort study of older people aged 65 years old and over, who were resident in Adelaide, South Australia. ALSA contains information on the health and wellbeing of 2087 participants. Frailty measures that were used in the analysis included two multidimensional measures (a frailty index and a prognostic frailty score) and two unidimensional measures (a frailty phenotype index and a simplified frailty phenotype index).

Results: ALSA participants were aged between 65-103 years; majority of participants lived in the community (94%). The mean age was 78 years and the median number of medicines used was 3 (range 0-9). Across four measures, prevalence of frailty varied from 2% to 49%. 35% of participants were identified as frail by at least one measure and only 0.6% were classified as frail on all four measures. Among the frail groups, 53% to 73% used 4 or more medicines compared to only 28% to 36% in the non-frail groups.

Discussion: The variation in frailty status between different measures provide challenges for researchers to evaluate the safety and effectiveness of medicines use among the frail elderly.
Key Health Professionals’ views of prescribing resources for older patients
Paulina Stehlik¹, Jennifer L Marriott¹, Pèteris Dārziņš². CMUS, Monash University¹, Parkville, VIC; Eastern Health Clinical School campus, Monash University², Forest Hill, VIC. (Introduced by Paulina Stehlik, Monash University, Parkville, VIC)

Introduction. Prescribing resources aimed at improving the quality use of medicines in the aged are either too simple and do not address potential interactions when there are many diseases and many medications, or, when providing complex information, appear to be impractical to use during consultations.

Aims. To explore health professionals’ views on currently available geriatric medication management resources and to determine what health professionals consider makes medication management resources useful.

Methods. Purposive convenience sampling was used to recruit geriatricians, GPs and accredited pharmacists for one hour, individual, semi-structured interviews. Recruitment continued until data saturation was achieved. Themes were identified using NVivo9 software.

Results. Participants felt that currently available prescribing resources did not meet their needs when managing aged patients primarily due to lack guidance on how to deal with complex issues in aged people and lack of relevance to the Australian setting. Identified barriers to providing optimal care included: lack of access to appropriate literature; issues, such as lack of time, with contextualising vast amounts of new health information; and incomplete or fragmented healthcare records. Key components which make resources useful included clear formatting, simplicity, use of peer-reviewed evidence-based recommendations and ready electronic access via an easy to use interface.

Discussion. Current resources do not meet health professionals’ needs when they seek practical assistance for prescribing to older people with multiple medical problems.

Major medication discrepancies in patients with type 2 diabetes mellitus (T2DM), referred from primary care, to a tertiary ambulatory diabetes centre.
Madonna F Azzi¹, Maria I Constantino³, Lisa Pont², Margaret McGill³, Stephen Twigg³, Ines Krass¹ Faculties of Pharmacy¹, Nursing² and Medicine³, University of Sydney, Sydney, NSW, Royal Prince Alfred Hospital³, Sydney.

Introduction. Multiple medications are typically used to manage glycaemia and prevent/treat the comorbidities/complications of diabetes. Patients who transition between interfaces of care, are at risk of medication discrepancies - intended or unintended differences, between recorded medication regimens.

Aims. To identify, classify and determine predictors of medication discrepancies for T2DM patients, referred from primary care to the Royal Prince Alfred Hospital (RPAH) Diabetes Service.

Methods. A retrospective audit of a random sample of 300 adult patients, who attended the RPAH Diabetes Service between 01 January 2010, and 31 December 2011, was conducted. Rates/types of medication discrepancies were identified by comparing the medication list obtained via a structured nurse-patient interview (SNPI) with that in the GP referral letter.

Discussion. Medication discrepancies from primary to tertiary care were prevalent for patients referred to the RPAH Diabetes Service. Automated GP referral letters/inaccurate GP records may have contributed. This suggests the need for routine medication reconciliation in all transitions of care to clarify the correct regimen, ensure efficacy and patient safety.
**High-throughput assay for simultaneous quantification of the plasma concentrations of morphine, fentanyl, midazolam and their major metabolites using automated SPE coupled to LC-MS/MS**

Sussan Ghassabian¹, Seyed Mojtaba Moosavi¹, Kiran Shekar², John F Fraser², Maree T Smith¹. CIPDD, Univ of Queensland, Brisbane, QLD¹; Critical Care Research Group, The Prince Charles Hosp, Univ of Queensland, Brisbane, QLD; School of Pharmacy, Univ of Queensland, Brisbane, QLD.

**Introduction:** A high throughput assay was required to measure the plasma concentration of morphine, fentanyl, midazolam, and their major metabolites; morphine-3-β-D-glucuronide (M3G), morphine-6- β-D-glucuronide (M6G), norfentanyl, 1'-hydroxymidazolam and 4-hydroxymidazolam, in plasma samples collected from critically ill patients receiving extracorporeal membrane oxygenation (ECMO).

**Aims:** To develop and fully validate an LC-MS/MS method utilising on-line robotic SPE to extract and quantify the plasma concentrations of the analytes of interest in human plasma.

**Methods:** Aliquots (150 μl) of human plasma and of a mixture of two internal standards, morphine-d3 (200 ng/mL) and 1'-hydroxymidazolam-d5 (50 ng/mL) in 50 mM ammonium acetate buffer (pH 9.25) were mixed and loaded onto polymeric SPE cartridges which were washed using 10% methanol in 50 mM ammonium acetate buffer, pH 9.25, before elution with mobile phase comprising 0.1% formic acid in water, and acetonitrile with a flow rate of 0.6 mL/min using an 11.5 min run time. The analytes were separated on a C18 X-Terra® analytical column.

**Results:** The linear concentration ranges were 0.5-100 ng/mL for fentanyl, norfentanyl, and midazolam; 1-200 ng/mL for 4-hydroxymidazolam, 2.5-500 ng/mL for 1'-hydroxymidazolam and 3.5-700 ng/mL for morphine, M3G and M6G. The method showed within-run and between-run precision (RSD and accuracy <20%) for quality control samples. Moreover, analytes were stable for at least 48h in the autosampler (except for 4-hydroxymidazolam which decreased by 22% after 24h), 5 h at room temperature and after three cycles of freeze and thaw. The absolute recovery was in the range 40% (midazolam) to 110% (morphine).

**Discussion:** The developed assay is convenient by minimising sample volume (150 μl), replacing at least 3 separate assays, eliminating manual solvent handling, evaporation and reconstitution steps used in previously reported methods. This assay is now being successfully utilised for an international, multi-centre, clinical study investigating pharmacokinetic changes during ECMO.

---

**Prescribing in the elderly – cytochrome P450 (CYP) enzyme inhibitors and substrates**

Karen P Kerr¹, Karen E. Mate¹,², Dimity Pond², Parker J. Magin², Nigel Stocks³, John Marley², Peter Disler². School Biomed Sci and Pharmacy², Univ of Newcastle, Newcastle, NSW; School of Med and Pub Health², Univ of Newcastle, Newcastle, NSW; School of Pop Health and Clin Practice³, Univ of Adelaide, SA; School of Rural Health⁴, Monash Univ, Melbourne, VIC.

**Introduction:** Drugs that are CYP enzyme inhibitors may increase the concentration of drugs that are CYP enzyme substrates and the risk of their adverse effects. Given that the elderly tend to be on more medications than younger patients, there is a greater chance for the occurrence of such drug interactions.

**Aims:** The aim was to determine whether CYP enzyme inhibitors and substrates are being co-prescribed.

**Methods:** 1,076 patients, aged 75 years or older, were recruited at four locations (Newcastle, Sydney, Melbourne, and Adelaide) in 2006-2009. A table of clinically relevant CYP drug interactions was used to search for the co-prescription of strong or moderate CYP inhibitors and their relevant substrates (Flockhart, 2007).

**Results:** There were 2 instances of strong CYP2D6 inhibitors (causes a > 80% decrease in substrate clearance - paroxetine and fluoxetine) being co-prescribed with a CYP2D6 substrate. Paroxetine (40 mg daily) was co-prescribed with flecainide (50 mg daily). Fluoxetine (20 mg daily) was co-prescribed with metoprolol (200 mg daily). Ten patients were prescribed verapamil, a moderate CYP3A4,5,7 inhibitor (causes a 50-80% decrease in substrate clearance) and the substrates for this enzyme, simvastatin (8 patients) or atorvastatin (2 patients). 29 patients were prescribed diltiazem, also a moderate CYP3A4,5,7 inhibitor and simvastatin (14 pts) or atorvastatin (15 patients). No patients on verapamil or diltiazem were co-prescribed pravastatin or rosuvastatin, which are not CYP3A4,5,7 substrates. All 14 patients were taking a higher than manufacturer recommended dose of simvastatin (10 mg) for the diltiazem/simvastatin combination and 6/8 patients for the verapamil/diltiazem combination.

**Discussion:** The degree of clinical relevance of these interactions will vary between patients and should be assessed on a case to case basis. This highlights the value of medication reviews.

**SPEAKER ABSTRACTS**

**Ion chromatographic separation and isolation of oligosaccharides of intact low-molecular-weight heparin for the determination of their anticoagulant and anti-inflammatory properties**

Madhur Shastri\(^1\), Cameron Johns\(^2\), Joseph P. Hutchinson\(^2\) and Rahul Patel\(^1\). School of Pharmacy, Univ of Tasmania\(^1\), Hobart, TAS. Australian Centre for Research on Separation Science, Univ of Tasmania\(^2\), Hobart, TAS.

**Introduction.** It is well-known that enoxaparin, a widely used anticoagulant and low-molecular-weight heparin (LMWH) containing a large number of oligosaccharides, possesses anti-inflammatory activity. While enoxaparin has shown promising results in various inflammatory disorders, some of its oligosaccharides have anti-inflammatory properties and others increase the risk of bleeding due to their anticoagulant effects.

**Aims.** To develop an effective ion-chromatographic (IC) technique which allows the separation, isolation and consequently the identification of different oligosaccharides of enoxaparin with or without anticoagulant activity.

**Methods.** Separations were performed on a semi-preparative CarboPac PA100 column (250 × 9 mm). The optimised NaCl eluent gradient was: 0-70 min: gradient from 32-74% 2 M NaCl in Milli-Q water (0.64-1.48 M NaCl). Total flow rate of 2.0 mL/min was maintained and UV detection at 232 nm was performed.

**Results.** The method successfully resolved enoxaparin into more than 30 different peaks. IC-derived oligosaccharides with high, moderate, low or no anticoagulant activity were identified using an anti-factor Xa assay. The anti-inflammatory activity of selected oligosaccharides was investigated using the Griess assay. Using this technique, the oligosaccharides of enoxaparin with low or no anticoagulant activity, whilst exhibiting significant anti-inflammatory activity, could be fractionated.

**Discussion.** In the present study, structurally complicated enoxaparin was successfully fractionated using a newly-developed IC method. An important application of this method was demonstrated by investigating the anti-inflammatory effects of its oligosaccharides. A number of studies have investigated the anti-inflammatory effects of intact enoxaparin. However, to our knowledge this is the first study demonstrating the anti-inflammatory effect of enoxaparin oligosaccharides obtained without prior chemical or enzymatic modification of the parent LMWH. This technique can provide a platform to identify the oligosaccharides which are devoid of significant anticoagulant activity and are responsible for the therapeutic effects of enoxaparin that have been observed in various inflammatory conditions.
**The Physicochemical Stability of Ceftazidime or Cephazolin in Peritoneal Dialysis Fresenius Infusion Bag**

Siti Farahwahida Shikh Mohd Fadzilah\(^1\), Rahul Patel\(^1\), Madhur Shastri\(^1\). School of Pharmacy, Univ of Tasmania\(^1\), Hobart, TAS; Pharmacy Dept, Royal Hobart Hosp\(^2\), Hobart, TAS; Australian Centre for Research on Separation Science, Univ of Tasmania\(^3\), Hobart, TAS.

Introduction. Fresenius balance is relatively new types of infusion bags containing dialysis solution used for patients with renal failure. This solution contains low-glucose degradation products which prolongs the function of the peritoneal membrane, thereby yielding better patient outcomes. Ceftazidime or cephazolin is often used for the treatment of Gram-negative or Gram-positive peritonitis in peritoneal dialysis (PD) patients. However, the stability of these antibiotics in Fresenius balance solution has not been investigated thus far.

Aim. To investigate the physico-chemical stability of ceftazidime or cephazolin in Fresenius balance peritoneal dialysis solution under two different storage temperatures.

Methods. Ceftazidime (500 mg/mL) or cephazolin (500 mg/mL) was injected into Fresenius PD bag to obtain the concentration of 500 mg/L. Bags (n=3) containing either ceftazidime or cephazolin were then stored at 4°C or 25°C. Each PD bag was removed from its respective storage condition and an aliquot (approximately 2 mL) was withdrawn on days 0, 1, 2, 3, 4, 5, 7, 14, 21 and 28. The concentration of each antibiotic before and after storage was determined by stability indicating high-performance liquid chromatography.

Results. Ceftazidime or cephazolin lost more than 90% of its initial concentration within 24 hours when stored at 25°C. On the other hand, both the antibiotics retained more than 95% of the initial concentration when kept at 4°C.

Discussion. Ceftazidime or cephazolin antibiotic admixtures in Fresenius balance peritoneal dialysis solution may be prepared in advance and stored at 4°C for at least 14 days avoiding the necessity for frequent preparation.

---

**The Physicochemical Stability of Diluted Iron Polymaltose in Polyvinyl Chloride Infusion Bags**

Rahul P Patel\(^1\), Troy Wanandy\(^2\), Serena Loring\(^1\), Cameron Johns\(^3\), Joseph Hutchinson\(^1\) and Madhur Shastri\(^1\). School of Pharmacy, Univ of Tasmania\(^1\), Hobart, TAS; Pharmacy Dept, Royal Hobart Hosp\(^2\), Hobart, TAS; Australian Centre for Research on Separation Science, Univ of Tasmania\(^3\), Hobart, TAS.

Introduction. The iron polymaltose (IPM) for intravenous infusion is commonly used iron therapy for patients with iron deficiency anaemia. It is prepared by diluting commercially available IPM injection (318 mg/2 mL) with 0.9% sodium chloride. The stability of extemporaneously prepared IPM is unknown beyond 24 hours. Therefore, such preparations must be prepared on daily basis which results in substantial limitations and problems.

Aims. To investigate the physico-chemical stability of diluted IPM over a 28-day period under different storage conditions.

Methods. The IPM infusion samples (2 mg/mL) were prepared under aseptic conditions and kept in light protective bags or exposed to artificial light and stored up to 28 days at either 4 or 25°C. Aliquots were withdrawn on days 0, 1, 2, 3, 6, 7, 14, 21 and 28. Samples were analysed by size-exclusion chromatography (SEC) to measure the changes in concentration or molecular weight of IPM before and after storage. Samples were also investigated for the particle size distribution by dynamic light scattering (DLS) and for the free iron (III) content by ion chromatography (IC). Furthermore, samples were visually inspected for discoloration, clarity and precipitation, and the pH values of samples were also measured.

Results. Visual, HP-SEC, DLS and IC analyses showed that IPM (2 mg/mL) in 0.9% sodium chloride for intravenous infusion prepared under aseptic conditions remained physically and chemically stable at room temperature (with or without light exposure) or in refrigerator for at least 28 days.

Discussion. This is the first study that investigated the physicochemical stability of diluted IPM. The results indicate that assigning the 28 days stability of IPM diluted in 0.9% sodium chloride and stored at room temperature or in a refrigerator would be appropriate.
A practical synthesis of D-rhamnose building blocks for synthetic bacterial O-polysaccharide conjugate vaccines
Matt S Zunk, Milton J Kiefel School of Pharmacy and Institute for Glycomics, Griffith University, Gold Coast, QLD

Bacterial cell surface glycans play an important role in infectious diseases. As our general knowledge of the importance of glycans in infectious diseases grows, so do efforts directed towards understanding the role of specific components within these cell surface glycoconjugates. In this regard, 6-deoxy-D-hexoses have been receiving increased attention, most notably because of their role in infectious diseases as key components associated with pathogenic bacteria.[1]

One example of these rare sugars in bacterial pathogenesis is D-rhamnose and its 4-deoxy derivatives, which are common components of the LPS and EPS from many human and plant pathogenic species. However, importantly due to the fact that D-rhamnose is exclusively found in microorganisms and not in mammals or plants, this rare bacterial monosaccharide is a promising target for the development of new anti-infective agents, practically synthetic bacterial O-polysaccharide conjugate vaccines.[2]

Although the synthesis of D-rhamnose (and D-rhamnosides) have been reported numerous times, it has been noted by many that the synthesis of selectively functionalised D-rhamnose derivatives has been hampered by lack of ready access to D-rhamnose in large quantities [3]. This presentation will discuss our efforts towards the synthesis of selectively functionalized D-rhamnose derivatives, and will describe our successful strategy that allows for the quick and efficient synthesis of D-rhamnose derivatives. To the best of our knowledge the approach we have developed is the most efficient and high yielding synthesis of D-rhamnose reported to date.


Glutamate efflux from brain to blood - Transport and uptake studies in a bovine in vitro blood-brain barrier model
Hans C Helms1, Rasmus Madelung1, Simon Groth1, Helle S. Waagepetersen2, Carsten U. Nielsen1, Birger Brodin1. Dept. of Pharmacy, University of Copenhagen 1, Copenhagen, DENMARK; Dept. of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, DENMARK. (introduced by Birger Brodin, University of Copenhagen, Copenhagen, DENMARK).

Introduction. The concentration of the excitotoxic amino acid, L-glutamate, in brain interstitial fluid is tightly regulated by uptake transporters and metabolism in astrocytes and neurons. Recent studies suggest that the blood-brain barrier may play a role in brain glutamate homeostasis.

Aims. The goal of the study was to investigate whether the blood-brain barrier plays a role in brain glutamate homeostasis.

Methods. Transendothelial transport- and accumulation studies of 3H-L-glutamate, 3H-L-aspartate and 3H-D-aspartate were performed in a tight bovine endothelial/rat astrocyte blood-brain barrier co-culture model. Results. The co-cultures displayed transendothelial resistance values of 1014 ± 70 ȍ·cm2, and 14C-D-mannitol permeability values of 0.88 ± 0.13 x 10^-6 cm · s-1 after six days of culture. Unidirectional flux studies showed that L-aspartate and L-glutamate, but not D-aspartate, displayed polarized transport in the brain-to-blood direction, however all three amino acids accumulated in the co-cultures when applied from the abluminal side. The transcellular transport kinetics were characterized with a K_m of 69 ± 15 μM and a J_max of 44 ± 3.1 pmol · min⁻¹ · cm⁻² for L-aspartate and a K_m of 138 ± 49 μM and J_max of 28 ± 3.1 pmol · min⁻¹ · cm⁻² for L-glutamate. The EAAT inhibitor, DL-threo-benzoyloxyaspartate, inhibited transendothelial brain-to-blood fluxes of L-glutamate and L-aspartate. Expression of EAAT-1 (Slc1a3), -2 (Slc1a2) and -3 (Slc1a1) mRNA in the endothelial cells was confirmed by conventional PCR and localization of EAAT-1 and -3 in endothelial cells was shown with immuno fluorescences. Abluminal uptake studies demonstrated that EAAT1 was responsible for uptake from the abluminal solution into the brain endothelial cells.

Discussion. The present study indicate that the blood-brain barrier effluxes glutamate from brain to blood via abluminal uptake via EAAT1 and luminal exit via a not yet characterized carrier system. Overall transport kinetics indicate that the efflux system may play a role under pathophysiological conditions.
The role of NOX2 NADPH oxidase in macrophage polarisation

Caitlin V Lewis, Elizabeth Guida, Christopher G Sobey, Grant R Drummond, Barbara K Kemp-Harper. Dept Pharmacology, Monash University, Clayton, VIC

Introduction. Advanced atherosclerotic plaques are associated with an increased ratio of pro-inflammatory M1 to anti-inflammatory M2 macrophages and increased activity of the reactive oxygen species generating enzyme, NOX2 NADPH oxidase. Given NOX2 deletion reduces atherosclerotic lesion size (Judkins et al., 2010), we hypothesized that NOX2 may contribute to the atherogenic actions of M1 macrophages and promote M1 polarisation.

Aims. To establish an in vitro model of macrophage polarisation and determine the role of NOX2 in M1 polarisation.

Methods. Phorbol-12,13-dibutyrate (PDBu, 10nM, 24hrs) -differentiated human monocytes (THP-1) were polarised toward either a M1 (lipopolysaccharide 10ng/ml + interferon-gamma 5ng/ml; LPS-IFN-γ, 72hrs) or a M2 (interleukin-4 25ng/ml; IL-4, 72hrs) phenotype. Quantitative real-time PCR was used to measure expression of M1 (CCR7, CXCL11) and M2 (MRC1) markers, and NADPH oxidase subunits. L012-enhanced chemiluminescence was used to measure PDBu (NOX2-derived) and ionomycin (NOX5-derived)-stimulated superoxide production.

Results. LPS/IFN-γ treatment selectively increased expression of M1 markers CXCL11 (50-fold, n=6; P<0.01) and CCR7 (250-fold, n=4; P<0.01) in THP-1 cells, whereas IL-4 treatment increased MRC1 expression (4-fold, n=6; P<0.05). M1 polarisation was associated with upregulation of NOX2 (4-fold, n=6; P=0.05) and its regulatory subunit, p47phox (7-fold, n=6; P<0.01), as well as an increase in PDBu-stimulated superoxide production. NOX2 expression was not elevated in M2-polarised cells, but expression of another isoform, NOX5 (3-fold, P<0.05) was upregulated. Consistent with this, ionomycin-stimulated superoxide production was elevated in M2 macrophages. Finally, chronic treatment with the NOX2 inhibitor, apocynin, did not prevent M1 macrophage polarisation.

Discussion. M1 and M2 macrophages are associated with increased NOX2 and NOX5 activity, respectively. While NOX2 inhibition may be a viable therapeutic strategy to limit the damaging effects of M1 macrophages, it may not lead to a change in polarisation state.

An investigation of the anti-inflammatory role of nitroxyl (HNO) on the endothelium.
Karen L Andrews1*, Amanda K Sampson1*, Chloe XE Lim1**, Natalie G Lumsden1, Barbara K Kemp-Harper2, Jaye PF Chin-Dusting1. Vascular Pharmacology, Baker IDI Heart and Diabetes Institute1, Melbourne, VIC, Department of Pharmacology, Monash University2, Clayton, VIC. * indicates joint first authorship

Introduction. Nitric oxide (NO*) interferes with key events in plaque development, including endothelial-leukocyte adhesion. Unfortunately, the use of the NO* donor, glyceryl trinitrate (GTN) as a treatment for cardiovascular disease is limited due to its susceptibility to tolerance and clearance by superoxide. The reduced congener of NO*, nitroxyl (HNO) is resistant to tolerance, yet the anti-inflammatory potential of HNO is unknown.

Aims. To assess the effects of the HNO donor, Angeli’s Salt (AS) compared to GTN, on endothelial inflammation.

Methods & Results. Both AS and GTN attenuated endothelial-leukocyte adhesion of monocytes (THP-1) to TNFα-activated HUVECs in a concentration-dependent manner (TNFα (10ng/mL); 100±6%, TNFα+AS (10μM); 36±4%, TNFα+GTN (10μM); 46±8%; n=3-4; P<0.001). The effects of AS and GTN were diminished with the soluble guanylate cyclase (sGC) inhibitor, ODQ (10μM; n=3; P<0.01), and GTN, but not AS, with the NO* scavenger, hydroxocobalamin (100μM; n=3; P<0.05). Flow cytometry analysis revealed that the protein expression of intercellular adhesion molecule-1 (ICAM-1) on TNFα-stimulated HUVECs was significantly reduced by AS (10μM; n=3; P<0.001), but not by GTN (P>0.05). In proof of concept experiments using mouse aorta stimulated with TNFα, both AS and GTN also attenuated adhesion of leukocytes from human blood compared to TNFα alone-treated vessels after 10 mins (TNFα; 18±2, TNFα+AS (10μM); 4±1, TNFα+GTN (10μM); 5±1 leukocytes/field; n=5-10; P<0.001). The effects of AS were abolished by the HNO scavenger, L-cysteine (3mM; n=3; P<0.001), and ODQ (n=3; P<0.01), and GTN by hydroxocobalamin (n=3; P<0.001). Aortic levels of ICAM-1, monocyte chemotactic protein-1 (MCP-1) and interleukin-6 (IL-6) mRNA were increased by TNFα (n=4-6; P<0.05) but were unaffected by either AS or GTN (n=3-6; P>0.05) suggesting post-transcriptional activity.

Discussion. These results demonstrate, for the first time, that AS reduces inflammation through an endothelial sGC-dependent mechanism and therefore may be a viable therapeutic agent in the treatment of cardiovascular disease.

Differential roles of KCNQ4 and KCNQ5 potassium channels in cerebral artery reactivity
Preet S Chadha1, Thomas A Jepps1, Iain A Greenwood1. Division of Biomedical Sciences, St George’s, University of London1, London, U.K. (introduced by Preet S Chadha, St George’s, London, U.K).

Introduction. Voltage-dependent potassium channels encoded by KCNQ genes (Kv7) participate in the myogenic control of cerebral artery diameter but there is no information on the role of individual isoforms in either myogenic activity or receptor-mediated vasorelaxation.

Aims. This study aims to define the specific contribution of Kv7.4 and Kv7.5 channels to myogenic and calcitonin gene-related peptide (CGRP) mediated regulation of cerebral artery diameter.

Methods. Potassium channel modulators and small interfering RNA (siRNA) strategies were allied with isometric and isobaric myography to investigate the contribution of Kv7.4 and Kv7.5 subtypes to CGRP-evoked dilation and pressure-induced myogenic constriction in middle cerebral arteries (MCA).

Results. Dilations to CGRP in precontracted MCA were inhibited by the pan-Kv7 channel blocker linopirdine (Emax: 43.7±9.8% compared to control 113.1±12.3%, n=6, P<0.01). Similarly, arteries depleted of KCNQ4 using selective siRNA were markedly less responsive to CGRP (Emax: 31.4±15.3% compared to scrambled siRNA 90.8±6.7%, n=5-8, P<0.05) and the Kv7 channel activator S-1 (P<0.01). In contrast, responses to CGRP and S-1 in arteries incubated with KCNQ5 siRNA were not different from scrambled siRNA transfections. However, downregulation of Kv7.5 channels by targeted siRNA markedly increased active myogenic constriction at nearly all pressures including the physiological range (40 to 80 mmHg).

Discussion. The present study identifies differential roles for Kv7 subtypes in the control of cerebral arterial tone. While Kv7.4 channels underlie a substantial portion of the dilation produced by CGRP, Kv7.5 channels predominate in the regulation of myogenic constriction.

**245**

**Hydrogen sulfide protects endothelial function under conditions of oxidative stress.**
Mohammad R Al-Magableh¹, Hooi Hooi Ng², Barbara K Kemp-Harper¹, Alyson A Miller¹ and Joanne L Hart².
Department of Pharmacology, Monash University¹, Clayton Victoria, School of Medical Sciences, RMIT University², Bundoora, Victoria.

Introduction. Hydrogen sulfide is an endogenously produced gas that is reported to have anti-oxidant effects.
Aims. The aim was to examine the capacity of the H₂S donor, NaHS to scavenge superoxide anions (O₂⁻) and to examine whether this effect elicited protection of endothelial function in oxidative stress.

Methods. O₂⁻ were generated in Krebs’ solution via the reaction of hypoxanthine (Hx 100μM) with xanthine oxidase (XO 0.01U/ml) or pyrogallol (PG 20μM). Thoracic aortae were collected from male C57Bl6/J mice and vascular reactivity and NO bioavailability were examined by myography. NADPH (100μM)-dependent lucigenin-enhanced chemiluminescence was used to examine the ability of NaHS to scavenge O₂⁻ with and without vascular tissue.

Results. NaHS scavenged O₂⁻ generated from Hx-XO in Krebs’ solution in a concentration-dependent manner (maximum reduction 59±4%, IC₅₀ 0.12μM, P<0.001). Aortic rings exposed to either Hx-XO or PG in the myograph displayed significantly attenuated vasorelaxation to the endothelium-dependent vasodilator acetylcholine (control: 90±4%, Hx-XO: 58.3 ± 4.0%, P<0.001) which was completely reversed by NaHS (100μM) or superoxide dismutase (250U/ml). Similarly, NO bioavailability was attenuated by PG (P<0.05), but restored by NaHS (100μM). NaHS treatment (100nM-100μM) for 30min inhibited vascular O₂⁻ generation in a concentration-dependent manner (maximum reduction 88±3%, IC₅₀ 2.4μM, P<0.001). This effect persisted when the aortic segments were incubated with NaHS (100nM-100μM) for 30min and the NaHS washed out before NADPH (100μM) was added (maximum reduction 58±7%, IC₅₀ 0.4μM, P<0.001).

Discussion. These data show that H₂S scavenges O₂⁻ generated via Hx-XO or PG and additionally inhibits NADPH-dependent O₂⁻ production. These properties protect endothelial function against oxidative stress in vitro.

**246**

**Carvedilol induces greater control by PDE3 of β₂- than β₁-adrenoceptor-mediated inotropic effects in human failing myocardium while PDE4 has no effect.**
Peter Molenaar¹, Torsten Christ², Rizwan I Hussain³, Andreas Engel², Emanuel Berk², Katherine T Gillette¹, Lu Chen¹, Alejandro Galindo-Tovar⁴, Kurt A Krobert³, Ursula Ravens², Finn Olav Levy³, Alberto J Kaumann⁵. Schl Biomed Sci, QUT¹, Brisbane; Schl Medicine, Uni QLD, Brisbane, QLD; Dept of Pharmacol and Toxicol, Dresden Uni. of Technology², Dresden, Germany; Dept of Pharmacol, Uni Oslo and Oslo Uni Hospital³, Oslo, Norway; Research Unit of Uni Hospital Virgen de la Arrixaca and Dept Pharmacol, Uni Murcia⁴, Murcia, Spain; Dept of Physiology, Development and Neuroscience, Uni Cambridge⁵, Cambridge, UK.

Introduction. Phosphodiesterases PDE3 and/or PDE4 control ventricular effects of catecholamines in several species but their relative effects in failing human ventricle are unknown.
Aims. To determine whether the PDE3-selective inhibitor cilostamide or PDE4 inhibitor rolipram modify the positive inotropic and lusitropic effects of catecholamines in human failing myocardium.

Methods. Ventricular trabeculae from freshly explanted hearts of 5 non-β-blocked, 11 carvedilol-treated and 15 metoprolol-treated patients with terminal heart failure were paced to contract at 1Hz. The effects of (-)-noradrenaline, mediated through β₁-adrenoceptors (β₂-adrenoceptors blocked with ICI118551), and (-)-adrenaline, mediated through β₂-adrenoceptors (β₁-adrenoceptors blocked with CGP20712A), were assessed in the absence and presence of PDE inhibitors.

Results. The positive inotropic and lusitropic effects of (-)-noradrenaline were potentiated 2-5-fold by cilostamide in metoprolol-treated but not in non-β-blocker-treated patients. Cilostamide caused 3-5-fold and 10-35-fold potentiation of the inotropic and lusitropic effects of (-)-adrenaline in metoprolol-treated and carvedilol-treated patients respectively. Rolipram did not affect the inotropic and lusitropic potencies of (-)-noradrenaline or (-)-adrenaline.

Discussion. Treatment of heart failure patients with carvedilol, and to a lesser extent with metoprolol, facilitates PDE3-induced reductions of the inotropic and lusitropic effects mediated through β₂-adrenoceptors. PDE4 had no effect.
Impact of the putative nitroxyl (HNO) donor 1-nitrosocyclohexyl acetate (1-NCA) on the intact rodent heart
Rebecca H Ritchie1, Yung G Wong1,2, Barbara K Kemp-Harper2, Nga Cao1. Heart Failure Pharmacology, Baker IDI Heart & Diabetes Institute1, Melbourne VIC; Dept of Pharmacology, Monash University2, Clayton, VIC.

Introduction. HNO (a redox sibling of NO) elicits vasodilator, antihypertrophic and superoxide-suppressing actions. HNO however is resistant to scavenging by superoxide, does not develop tolerance to its vascular actions, and is highly thiophilic. The impact of the putative mixed HNO/NO donor 1-NCA on left ventricular (LV) function however remain largely unresolved.

Aims. We tested the hypothesis that 1-NCA enhances LV function, and sought insight into its effectiveness in the context of diabetes-induced LV dysfunction.

Methods. The acute dose-response curve to 1-NCA (10⁻⁷-10⁻¹ mol) following U46619 preconstriction was determined in isolated adult male Sprague-Dawley rat hearts subjected to Langendorff perfusion in vitro, on coronary flow and LV function. In parallel studies, 1-NCA (83mg/kg/daily i.p. for 4wks) was administered to both normal and streptozotocin-diabetic adult male FVB/N mice (commencing after 4wks diabetes), with LV function determined on both echocardiography and LV catheterisation.

Results. Bolus 1-NCA doses significantly increased coronary flow (by 3.9±0.9ml/min, n=3 at the highest dose studied) in the normal rat heart in vitro. Although modest trends for enhanced LV function were evident, these were not significant. The HNO scavenger L-cysteine (4mM, n=7), but not the NO scavenger hydroxocobalamin (50μM, n=3), significantly blunted 1-NCA coronary vasodilatation. Chronic treatment with 1-NCA significantly reduced LV superoxide generation by 28±4% (P<0.0005, dihydroethidium fluorescence), but did not affect other measures of LV systolic or diastolic function in non-diabetic mice. In contrast, diabetes-induced impairments in LV diastolic function were significantly ameliorated by chronic 1-NCA, on both peak atrial (A) wave velocity and the ratio of peak early (E):A wave velocities, descriptors of LV filling (both P<0.05, n=4-7).

Discussion. In conclusion, 1-NCA elicits HNO-dependent coronary vasodilatation, accompanied by superoxide suppression. Its ability to enhance LV function in the intact heart may be selective for settings where diastolic function is impaired.

Compound 21, a synthetic AT2 receptor agonist, evokes neuroprotection in a conscious rat model of ischaemic stroke
Lachlan J Facey1, Jennifer K Callaway2, Robert E Widdop1 & Claudia A McCarthy2. 1Dept of Pharmacol, Monash Univ, Clayton, VIC. 2Dept of Pharmacol, Univ of Melbourne, Parkville, VIC.

Introduction: Central AT2 receptor stimulation using the peptide agonist CGP42112 has been shown to be neuroprotective (McCarthy et al 2009). The current study has used the non-peptide, Compound 21, as an AT2R agonist.

Aim: We hypothesised that the peripheral administration of Compound 21 would evoke neuroprotection in an ischaemic model of stroke.

Methods: Animals were anaesthetised with ketamine (75mg/kg; i.p) & xylazine (10mg/kg; i.p) for stereotaxic insertion of a guide cannula. Ischemia was induced in conscious rat hearts subjected to Langendorff perfusion in vitro, on coronary flow and LV function. In parallel studies, 1-NCA (83mg/kg/daily i.p. for 4wks) was administered to both normal and streptozotocin-diabetic adult male FVB/N mice (commencing after 4wks diabetes), with LV function determined on both echocardiography and LV catheterisation.

Results: When administered prior to stroke, Compound 21 and candesartan reduced cortical infarct volume compared to vehicle by approximately 58% and 54%, respectively (P<0.05 vs. vehicle), independent of any changes in blood pressure. Moreover, both treatments significantly improved behavioural deficit 24 hours after stroke. When Compound 21 was administered six hours after stroke (3 mg/kg, i.p. bolus), and continued for 3 days (0.3 mg/kg/day), it caused less neuroprotection than seen with pre-treatment. Compound 21 significantly increased neurite outgrowth (50.5% of cells expressing neurites, p<0.001 vs. vehicle), in PC12W cells compared to vehicle (13.5%), and this was abolished with the co-administration of PD123319.

Discussion: The current study has shown, for the first time that systemic administration of the novel AT2 receptor agonist, Compound 21, is as protective as AT1R blockade in a physiologically relevant conscious model of stroke.

The best of both worlds: creating an inclusive learning environment for experimental and clinical pharmacology
Dr Hilary Lloyd, The University of Sydney, Sydney, NSW

Changes in teaching methods (e.g. from traditional to integrated teaching), the diversity of degree programs into which pharmacology is taught and the expanding knowledge base of the discipline have all contributed to the challenges of teaching pharmacology. In 2008 - 2009, a national audit of pharmacology curricula in Australia was undertaken (Hinton et al., 2010). Five degree programs (science, medicine, pharmacy, nursing and allied-health) were surveyed for course content, teaching methods and assessment. Similar subject areas were found to be taught across the five degree programs supporting the view that a generic pharmacology curriculum (or core curriculum) can be defined for science and health-related degree programs. Differences between degree programs, however, were found with respect to the breadth and depth of teaching within a given subject area and the pedagogical approach used for teaching and learning. In order to harness these differences, whilst retaining common core content, it is proposed that specific learning outcomes and levels of attainment expected for each degree program are articulated alongside curriculum content to create a flexible curriculum framework. Such a proposal could provide a national pharmacology curriculum that can be tailored to specific degree requirements. It offers several advantages. Defining the broad knowledge base of pharmacology preserves discipline identity and assists in curriculum mapping; articulating learning outcomes and standards ensures that the requirements of each degree program will be met; and creating an inclusive learning environment facilitates the sharing of valuable expertise and innovative teaching practices to enhance the standard of teaching and learning across experimental and clinical pharmacology.


---

Much ado about NSAIDs
Kathleen M Knights. Department of Clinical Pharmacology, School of Medicine, Flinders University, Adelaide, SA

Although in clinical practice since 1899 the chameleon nature of nonsteroidal anti-inflammatory drugs (NSAIDs) continues to provide challenges. NSAIDs are a homogeneous group in terms of inhibition of the cyclooxygenase enzymes COX-1 and COX-2 but a chemically heterogenous group that includes derivatives of acetic, enolic, fenamic, propionic and salicylic acid and the diaryl heterocyclic COX-2 inhibitors. Metabolism is primarily hepatic, variably involving functionalisation and conjugation reactions. Early studies established that the hepatic metabolism of the R(-) enantiomers of the 2-arylpropionates involved formation of a coenzyme A (CoA) thioester intermediate, which following racemisation and subsequent hydrolysis, yielded the pharmacologically active S-enantiomer. Formation of the CoA conjugate was catalysed by a hepatic microsomal ATP dependent long-chain acyl CoA synthetase. Chiral inversion is the major determinant of (R)-ibuprofen clearance in humans and we established that gender and hormonal factors did not influence metabolism via this pathway. Subsequent studies characterising NSAID glucuronidation established the presence of enzymes of the UDP-glucuronosyltransferases superfamily UGTIA and UGT2B7 in the human nephron. We established that fatty acids were potent inhibitors of glucuronidation and that inclusion of albumin in microsomal incubations improves the accuracy of in vitro-in vivo extrapolation for substrates of UGT1A9 and UGT2B7. The common involvement of UGT2B7 in the metabolism of NSAIDs and aldosterone led us to screen NSAIDs as inhibitors of aldosterone glucuronidation. Determination of the inhibition constant of five NSAIDs established a rank order of potency of inhibition of aldosterone glucuronidation as mafenamic acid>diclofenac>naproxen>indomethacin>S-ibuprofen. Further we established that >3 months use of diclofenac was associated with greater arterial dysfunction in comparison to naproxen, indomethacin and ibuprofen thus alluding to a role in the cardiovascular toxicity of NSAIDs. Over the course of 113 years NSAIDs have been identified as gastrotoxic, nephrotoxic and now cardiovascular toxic drugs, is there more to come?
Teaching the ‘new’ prescribers: The challenges of knowledge, attitudes and skills
Kathleen M Knights1, Andrew Rowland1, John O Miners1 Department of Clinical Pharmacology, School of Medicine, Flinders University1, Adelaide, SA

The extension of prescribing rights to multiple groups of health care professionals other than medical practitioners started in the USA in 1965 when Nurse Practitioners emerged in response to a shortage of physicians in rural and disadvantaged communities. In the UK ‘nurse prescribing’ was adopted nationally in 1998 and all nurses with a district nursing or health visiting qualification were eligible for training to prescribe from the Nurse Prescribers Formulary. Nurse independent prescribing was introduced in the UK 2002 and this was followed shortly after by nurse and pharmacist supplementary prescribing (2003); radiographers, podiatrists, chiropodists, physiotherapists and optometrists supplementary prescribing (2005); pharmacist independent prescribing (2006) and optometrists independent prescribing (2008). In New Zealand midwives were granted prescribing rights in 1998 while in Australia the first Nurse Practitioner was appointed in NSW in 2001. The other Australian States followed with the authorisation of prescribing rights to Nurse Practitioners in Victoria in 2000 and South Australia in 2002. Since that time it has been identified that the greatest education challenges facing higher education providers involved in the education of the Nurse Practitioners include meeting the needs of diverse groups of students with varied interests, differing levels of prior knowledge and varied clinical backgrounds. Development of pharmacology curricula, assessment methods, determination of prescribing skills and competencies and the incorporation of flexible learning environments is all impacted by an overarching background of National Competency Standards for the individual health care professions, the National Prescribing Service “Prescribing Competencies Framework”, HealthWorkforce Australia’s “Health Professionals Prescribing Pathway Project” and TEQSA. The educational challenges for the Discipline of Pharmacology are enormous and teaching and learning is but one aspect of the challenge.

The devil is in the detail
R E Loiacono; Department of Pharmacology, School of Biomedical Sciences, Monash University, Clayton, VIC

Teaching Pharmacology across different courses presents some issues in setting the level of difficulty, depth and breadth. It also raises the idea of having different tasks of varying levels of cognitive complexity with matched activities across any one course; with low level activities relying on recall and recognition to high activities tasks that engage students in reasoning and analysis. While the low cognitive complexity activities are likely to be shared across courses, the high cognitive complexity activities are not. Moreover, the time devoted to each of these “depth of knowledge” activities could vary across different courses. Do we intrinsically “assign” different levels of depth of knowledge and parse the level of complexity across different courses? The level of recall and the basic application of pharmacological knowledge for medical students would be similar to science / biomedical science students; but, there would be divergence when it comes to extended thinking requiring the differentiation between a family of clinically used compounds to the detailed investigation of the pharmacodynamics characteristics of agents targeting a receptor system. Highly complex tasks for science / biomedical science students tend to construct models for research, while in clinical related courses such as medicine; students tend to solve a problem based on several alternatives finding the best outcome.
**SPEAKER ABSTRACTS**

**Myocardial stress and ischemic injury - sex and sex steroid influences**
L.M.D. Delbridge. Cardiac Phenomics Laboratory, Department of Physiology, The University of Melbourne, VIC

The occurrence of myocardial infarction in women and men is associated with differential clinical outcomes relating to sudden cardiac death and progression to failure. For women there is also evidence that coincident diabetes and underlying cardiac hypertrophy selectively and negatively modulate the severity of response to a clinical event. An understanding of the mechanistic bases for these differences is currently lacking. Experimentally at the level of the single cardiomyocyte, we have identified fundamental differences in the contractile performance and in the handling of activator Ca\(^{2+}\). We have also characterized sex differences in the ex vivo responses of isolated hearts to ischemic stress (Bell et al, 2008). These investigations suggest increased mechanical reserve in female hearts in association with upregulation of signalling through PI3K activation of Akt and also PKC activation of ERK1/2. When ischemic challenge is assessed in hearts which exhibit genetically-determined hypertrophy, these sex-specific response differences are blunted. Furthermore, when energy stress is induced to perturbs PI3K myocardial signalling, sex differences in the energy mobilization responses are observed. It is postulated that sex steroids play a role in determining/modulating sex-specific aspects of function, signalling and injury vulnerability. Recently we have reported that myocardial tissue expresses the enzyme aromatase - indicative of the capacity for local cardiac androgen-to-estrogen conversion. Our experiments with genetically manipulated rodents, where tissue aromatase expression has been suppressed or enhanced, provide evidence that altered sex-steroid conversion impacts on cardiac injury responses - including signalling, inotropy and arrhythmogenesis (Bell et al 2011). Further exploration of these sex-specific responses to myocardial stress will yield clinically relevant sex-targeted therapies for the ischemic heart.


**Y chromosome dependent blood pressure regulation in the SHRSP is mediated, in part, by the renal renin angiotensin system.**
Amanda K Sampson.
Vascular Pharmacology Department, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria.

Introduction. The Y chromosome influences blood pressure regulation with studies demonstrating introgression of the Y chromosome from a normotensive into a hypertensive rat strain reduces blood pressure by 10-15mmHg. One candidate gene implicated to contribute to the Y-chromosome dependent regulation of blood pressure is the Sry3 gene. It is located exclusively on the Y chromosome from the spontaneously hypertensive rat (SHR) and has been shown in vitro to interact with the renin angiotensin system (RAS); upregulating angiotensinogen, renin and ACE gene promoter activity. However, the functional consequence(s) of this interaction on renal function and renal RAS responses in vivo remains unknown.

Aims. We aimed to investigate the functional consequences of the interaction of the Y chromosome with the renal RAS in vivo using 16 week old normotensive (WKY), hypertensive (SHRSP) and 2 consomic strains; one in which the WKY Y chromosome was introgressed into the SHRSP background (SP.WKY GlaY) and vice versa (WKY.SPGlaY).

Results. Systolic blood pressure, measured by radiotelemetry, was lower in the SP.WKY GlaY vs SHRSP (195±5mmHg vs 227±8mmHg, n=8, P<0.03) and was higher in the WKY.SPGlaY vs WKY (157±5mmHg vs 148±3mmHg, n=8, P<0.05). The ratio of plasma Ang(1-7):Ang II was higher in the SHRSP when compared to all other strains (n=5, P<0.01). In addition, SHRSP had greater renal AT1R, AT2R and MasR mRNA gene expression (P<0.005 compared to WKY, n>6) which was not present in the SP.WKY GlaY. Renal blood flow responses to graded intrarenal bolus doses (1, 3, 10, 30, 100ng/kg) of Ang I and Ang(1-7) were blunted in the SHRSP when compared to both the SP.WKY GlaY and WKY (P<0.007 and P<0.008, respectively). Renal responses to intrarenal Ang II (1,3,10,30,100 ng/kg) were similar in all strains.

Discussion. This study provides novel evidence that the Y chromosome enhances the vasodilatory components of the RAS in the SHRSP which is restored following introgression of the WKY Y chromosome.
Gene-environment interactions and sexual dimorphism in mouse models of brain disorders
Terence Y. Pang1, Thibault Renoir1, Emma L. Burrows1, Mark I. Ransome1, Xin Du1, Christina Mo1, Annabel K. Short1, Mari Kondo1,2, Anthony J. Hannan1 Florey Institute of Neuroscience and Mental Health, Melbourne Brain Centre, University of Melbourne, 1 Parkville, VIC; Department of Psychiatry and Behavioural Sciences, Johns Hopkins University School of Medicine2, Baltimore, MD, USA.

Huntington’s disease (HD) is a tandem repeat (CAG) expansion disorder involving a triad of psychiatric, cognitive and motor symptoms. In a transgenic mouse model of HD we have demonstrated that environmental enrichment (which enhances sensory stimulation, cognitive activity and physical exercise) can delay onset of the affective, cognitive and motor endophenotypes. Detailed investigations of these HD mice have also revealed sexually dimorphic depression-like behaviours which precede cognitive and motor deficits. The female HD mice demonstrate early affective abnormalities which can be rescued by administration of clinically effective antidepressant drugs as well as increased physical exercise. This is consistent with the clinical sexual dimorphism in the incidence of depression. We have thus been able to investigate these mice as a model of depression and HD and have discovered various molecular abnormalities, including specific deficits in neurotrophin, serotonergic and dopaminergic signalling pathways. A selective subset of these molecular changes have been found to be sexually dimorphic and, along with a possible role of sex hormones, may help explain the depression-like behaviours in these female HD mice.

These findings have been extended to additional environmental factors (e.g. stress), neuroendocrine modulators (e.g. sex hormones) and animal models of other brain disorders. For example, we have characterized behavioural and molecular changes in knock-out and knock-in mice modelling autism spectrum disorder. These models involve X chromosome gene mutations and thus they exhibit strong sexual dimorphism, reflecting the high clinical incidence of autism in boys.

Together with epidemiological studies and clinical trials, our findings are informing mechanisms of pathogenesis and the subsequent design of future intervention studies for these sexually dimorphic brain disorders. The models of gene-environment interactions can also be used to identify novel molecular targets for ‘enviromimetics’, drugs which mimic or enhance the beneficial effects of environmental stimulation.

Sex differences in the role of the renin-angiotensin system in the regulation of arterial pressure and renal function.
Kate M Denton. Department of Physiology, Monash University, Melbourne, VIC.

Introduction: Women are protected from cardiovascular and renal disease relative to men, prior to menopause. The mechanisms are poorly understood, but evidence demonstrates that estrogen plays a protective role against cardiovascular disease in women. There are major sex-differences in the expression levels of components of the renin-angiotensin system (RAS) and also differences in the way males and females respond to stimulation and inhibition of the RAS under physiological and pathophysiological circumstances.

Our studies: We have demonstrated that the depressor RAS pathways are enhanced in females and that the angiotensin type 2 receptor (AT2R) has a vasodilatory role in the response to chronic angiotensin II (AngII) infusion in female but not male rats. This action of AngII to decrease arterial pressure in females was mediated via an AT2R, estrogen dependent, mechanism. Thus, the AT2R plays a role in countering the pressor actions of AngII at the angiotensin type 1 receptor in females. Importantly, no differences in acute pressor responses to AngII were observed between the sexes, rather the differences become apparent during chronic RAS activation in females. However, we have shown acute sex-differences in the contribution of the depressor arm of the RAS to the renal mechanisms that control extracellular fluid homeostasis (pressure-natriuresis; tubulo-glomerular feedback) and hence arterial pressure. Thus, RAS depressor mechanisms in the kidney, which promote salt and water excretion, confer protection from increases in arterial pressure in females.

Future perspectives: Our work now focuses upon the role of the RAS during pregnancy and in ageing, to determine if the RAS depressor pathways are potential therapeutic targets for the treatment of hypertension and renal disease.
SPEAKER ABSTRACTS

Understanding idiosyncratic adverse drug reactions through integrative systems approaches
Romano Fois; Fac of Pharm, University of Sydney, Sydney, NSW.

Idiosyncratic adverse drug reactions (ADRs) remain intractable problems that continue to challenge clinicians and the pharmaceutical industry. The severest manifestations can cause irreversible morbidity in individuals and death; often with little warning. The inherently rare nature of these events and the absence of reliable animal models or in-vitro methods to study the mechanisms behind these ADRs render their occurrence largely unpredictable. While a number of theories may surround many idiosyncratic ADRs, they often involve consideration of a restricted part of the broader biological network of interactions (e.g. the role of hERG potassium channel inhibition in Torsade de Pointes (TdP)). Such reductionist approaches have generated valuable information; however they often fall short in accurately predicting vulnerability in patients or risks from specific medicines. This paper explores principles of off-target drug action within biological networks - complex adaptive systems. The features of these systems can explain the limitations of reductionism in explaining the underlying mechanisms for these rare toxicities and in accurately identifying “at-risk” individuals. Integrative systems approaches seek to identify important features and interactions in biological networks that together may explain vulnerability to idiosyncratic ADRs. These approaches rely on the wealth of knowledge that resides in disparate and growing data repositories. The growth in power of computational and information technologies and the development of tools and processes that can link information across these datasets can identify previously-unrecognised patterns of interaction among drugs and components of biological systems at a number of levels and may reveal the roles of specific targets, biological pathways and risk factors involved in the development of drug toxicity. The combination of adverse reaction and drug (chemistry, pharmacokinetic and pharmacodynamic) information with knowledge of variability among genes, proteins and biological metabolites presents opportunities for understanding the factors that conspire to produce toxicity. Examples of integrative approaches to understanding drug toxicity will be presented together with our recent work that has linked human population pharmacovigilance data with computational chemistry data and ligand-protein interaction information to identify structural and biological components and potential biological pathways implicated in drug-induced liver injury, parasomnias and cardiac arrhythmia (TdP).
Care track as a methodology and relevance for drug safety
Richard Day1,2, St Vincent's Hospital Clinical School and Pharmacology1, UNSW; St Vincent's Hospital2, Sydney, NSW

The landmark CareTrack study revealed that in 2009-10 Australians received appropriate care for 22 common conditions accounting for 40% of the burden of illness 57% of the time (95% CI, 54%–60%) of 35,573 eligible health care encounters). The range across conditions was 13% (alcohol dependence) to 90% (coronary artery disease) and for individual practitioners with more than 300 encounters, 32% to 86%. These data indicate room for improvement. Many hypothesise that feedback of quality of care data to individual clinicians in respect of their own patients will lead to improvements in achieving minimal standards of care.

Many of the conditions such as heart failure and type II diabetes are chronic, heavily reliant on multiple medications and more often affect the elderly where adverse drug reactions and interactions and medication errors are more likely. Automatic data retrieval and checking against indicators of care using the general approach of the CareTrack study is possible with the advent of the electronic health record. Critical to the effort is a more systemised approach to developing and owning standards of care and associated indicators. These can then be used as tools that can be used to monitor and feedback quality of care to individual practitioners. Wider access to the standards of care not only to clinicians but also to their patients will drive improvement.

References
Runciman W, Coeira E, Day R et al. Towards the delivery of appropriate health care in Australia MJA 2012; 197 (2):78-81

Acknowledgements
Bill Runciman, Tamara Hunt, Natalie Hannaford, Peter Hibbert, Diane Hindmarsh, Elizabeth McGlynn, Enrico Coiera, Johanna Westbrook, Jeffrey Braithwaite

Injectable and oral contraceptive use and cancers of the breast, cervix, ovary, and endometrium in black South African women: case–control study
Emily Banks3,4, Margaret Urban1,2, Sam Egger5, Karen Canfell5,6, Dianne O'Connell5,6,7,8, Valerie Beral9, Freddy Sitas5,6,7 NHLS/MRC Cancer Epidemiology Research Group, National Health Laboratory Services1, Johannesburg, South Africa, Faculty of Health Sciences, University of the Witwatersrand2, Johannesburg, South Africa, National Centre for Epidemiology and Population Health, The Australian National University3, Canberra, Australia, The Sax Institute, Sydney4, Australia, Cancer Council New South Wales5, Sydney, Australia, Sydney Medical School—Public Health, University of Sydney 6, Sydney, Australia, School of Public Health and Community Medicine, University of New South Wales7, Sydney, Australia, School of Medicine and Public Health, University of Newcastle8, Newcastle, Australia, Cancer Epidemiology Unit, University of Oxford9, Oxford, United Kingdom

Background: Oral contraceptives are known to influence the risk of cancers of the female reproductive system. Evidence regarding the relationship between injectable contraceptives and these cancers is limited, especially in black South Africans, among whom injectable contraceptives are used more commonly than oral contraceptives.

Methods and Findings: We analysed data from a South African hospital-based case–control study of black females aged 18–79 y, comparing self-reported contraceptive use in patients with breast (n = 1,664), cervical (n = 2,182), ovarian (n = 182), and endometrial (n = 182) cancer, with self-reported contraceptive use in 1,492 control patients diagnosed with cancers with no known relationship to hormonal contraceptive use. We adjusted for potential confounding factors, including age, calendar year of diagnosis, education, smoking, alcohol, parity/age at first birth, and number of sexual partners. Among controls, 26% had used injectable and 20% had used oral contraceptives. For current and more recent users versus never users of oral or injectable contraceptives, the odds ratios (ORs) for breast cancer were significantly increased in users of oral and/or injectable contraceptives (OR 1.66, 95% CI 1.28–2.16, p = 0.001) and separately among those exclusively using oral (1.57, 1.03–2.40, p = 0.04) and exclusively using injectable (OR 1.83, 1.31–2.55, p < 0.001) contraceptives; corresponding ORs for cervical cancer were 1.18 (1.08–1.28, p = 0.01), 1.01 (0.66–1.56, p = 0.96), and 1.58 (1.16–2.15, p = 0.004). There was no significant increase in breast or cervical cancer risk among women ceasing hormonal contraceptive use ≥10 y previously (p = 0.3 and p = 0.9, respectively). For durations of use ≥5 y versus never use, the ORs of ovarian cancer were 0.60 (0.36–0.99, p = 0.04) for oral and/or injectable contraceptive use and 0.07 (0.01–0.49, p = 0.008) for injectable use exclusively; corresponding ORs for endometrial cancer were 0.44 (0.22–0.86, p = 0.02) and 0.36 (0.11–1.26, p = 0.1).

Conclusions: In this study, use of oral and of injectable hormonal contraceptives was associated with a transiently increased risk of breast and cervical cancer and, for long durations of use, with a reduced risk of ovarian and endometrial cancer. The observed effects of injectable and of oral contraceptives on cancer risk in this study did not appear to differ substantially.
Pharmacometrics methods to individualise dose
Stefanie Hennig, School of Pharmacy, University of Queensland, Brisbane, QLD.

Pharmacometrics has been defined as “the science of developing and applying mathematical and statistical methods to: characterize, understand, and predict a drug’s pharmacokinetic and pharmacodynamic behaviour; quantify uncertainty of information about that behaviour, and rationalize data-driven decision making in the drug development process and pharmacotherapy.” The approach has been shown to be useful over the years to not only guide decision making in drug development, but more so in ‘getting the dose right’ in the clinical setting. The research presented here has mainly focused on the later and will showcase how the pharmacometrics approach can help find the right dose for therapeutic subgroups such as children and patients with cystic fibrosis. At the same time it will also be shown how using a model-based approach can enhance study design. In particular these topics have been looked at for designing studies with sparse sampling, under clinical restrictions, for therapeutic drug monitoring purposes or for drugs with a narrow therapeutic index. Lastly, I will present research into improving and enhancing the understanding of the currently used methods and clinical trial designs. Further efforts have been made in the last years to support the application of this methodology through teaching and facilitating of user-friendly software.


Preventing resistance of bacterial “superbugs” by synergistic combinations of available antibiotics
Jürgen B Bulitta, Centre for Medicine Use and Safety, Monash Univ, Parkville, VIC. (introduced by Carl MJ Kirkpatrick, Monash Univ, Parkville, VIC).

Introduction. Resistant bacterial ‘superbugs’ present one of the three most serious threats to global health. As this severe problem is exacerbated by a long-term lack of new antibiotics, novel strategies to rationally optimise combinations of available antibiotics are very promising to combat this global health crisis. Pseudomonas aeruginosa is one of the most problematic Gram-negative ‘superbugs’ and has an exceptional capacity to become resistant during therapy.

Aims. To develop innovative strategies how to identify, prospectively optimise, and rationally translate synergistic antibiotic combinations that maximise bacterial killing and prevent resistance via latest experimental and mechanism-based mathematical modelling approaches.

Methods. In vitro time-kill studies assessed combinations of two β-lactam antibiotics or of a β-lactam and an aminoglycoside antibiotic with different receptor occupancy patterns against wild-type and hypermutating P. aeruginosa strains. Viable counts of susceptible and ‘resistant’ bacteria were quantified in static time-kill studies over 48 h. Antibiotic dosage regimens were prospectively optimised in dynamic hollow fibre in vitro infection models over 10-days. Antibiotic concentrations were determined by LC-MS/MS. Novel, mechanism-based mathematical models accounted for specific receptor occupancy patterns and resistance mechanisms.

Results. Combinations of β-lactam antibiotics binding penicillin-binding proteins (PBPs) 1 to 4 achieved substantial and synergistic killing against a high inoculum of P. aeruginosa, whereas monotherapy with all tested anti-pseudomonal cephalosporins achieved limited or no killing over 48 h. Optimal combinations of β-lactams with different resistance mechanisms achieved synergistic killing without resistance in 10-day hollow fibre models. Imipenem binds all PBPs in P. aeruginosa and led to rapid killing, but also to non-replicating persisters which could be eradicated without resistance by double β-lactam combinations or tobramycin.

Discussion. Synergistic combinations of available and safe antibiotics were prospectively optimised to maximise bacterial killing and prevent resistance via latest experimental and mechanism-based mathematical models. This approach holds excellent promise to combat resistant bacterial ‘superbugs’.
Positive and negative outcomes from medicines in older adults  
Danijela Gnjidic1,2. Faculty of Pharmacy, Univ of Sydney1, Sydney, NSW; Sydney Medical School, Univ of Sydney2, Sydney, NSW.

Introduction. In older adults, evidence on medicines exposure and risks is commonly obtained from post-marketing studies. Aims. To discuss the role of pharmaco-epidemiological studies in determining positive and negative medicine-related outcomes in older adults. Methods. Pharmacoepidemiology employs epidemiological methodologies to study the utilisation and effects (beneficial or adverse) of medicines in large populations. Observational studies commonly utilise data from large cohort studies, healthcare and clinical databases, and drug and disease registries. Results. In older populations, pharmaco-epidemiological studies are essential to assess the medicine-related adverse outcomes, and to evaluate efficacy of medicines in real-world settings. This is because the representation and representativeness of older people in published randomised clinical trials is generally poor. However, quantification of causality requires judicious interpretation of observational data. For instance, pharmaco-epidemiological studies can be influenced by the study population’s characteristics, and their country or region’s system of health care. Therefore, testing the hypotheses across different countries with different health systems is essential to achieve external validity and generalisability. Discussion. Observational studies are critical in quantifying medication-related outcomes in older adults. Where possible, findings of observational studies should be tested with pragmatic real-world interventional trials specifically designed for older adults.

Allosteric modulation of G protein-coupled receptors  
Celine Valant, Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Parkville, VIC.

It is now well established that virtually all G protein-coupled receptors (GPCRs) possess topographically distinct allosteric binding sites that can be targeted to modulate the activity of orthosteric ligands. Accordingly, recent years have seen a dramatic increase in the discovery of allosteric GPCR modulators. A key challenge to the field is the means to optimally describe allosteric effects in a manner that can capture experimentally observed observations and facilitate enriched structure-activity studies and/or inform drug candidate selection matrices. One approach to this challenge is to assign numbers to allostery using operational modeling. Such models describe GPCR allosterism minimalistically in terms of modulator affinity ($K_a$) for the free receptor, modulation of the binding ($\alpha$) and/or signaling ($\beta$) of the orthosteric ligand, and intrinsic agonism ($\tau_0$) of the allosteric modulator itself; it is apparent that most allosteric ligands are likely to display mixtures of these properties in a cell-dependent manner. Other recent paradigms that have emerged from the study of GPCR allostery are the concepts of allosteric ligand signaling bias (functional selectivity) and probe-dependence, with both having major consequences for novel drug discovery programs. Additionally, amongst the growing categories of ligands for GPCRs, a novel type of ligand has emerged, the bitopic ligand, i.e., compounds composed of distinct orthosteric and allosteric pharmacophores joined by an appropriately chosen linker. An advantage of such ligands is the ability to ensure receptor activation/inactivation through an appropriately chosen orthosteric moiety, while inducing either subtype and/or functional selectivity through the allosteric moiety. With the recent solution of multiple Family A GPCR crystal structures, the possibility of more rational exploitation of novel binding pockets either above or below the orthosteric site promises to facilitate true structure-based drug discovery for allosteric GPCR ligands.
Effect of an educational workshop on pharmacists’ knowledge, attitudes and beliefs towards low back pain (LBP).
Christina Abdel Shaheed1, Chris G Maher2,3, Wendy Mak2,3, Kylie Williams4, Andrew J McLachlan1 Faculty of Pharmacy, University of Sydney, NSW1 The George Institute for Global Health, Sydney, NSW2, Sydney Medical School, University of Sydney, NSW3, School of Pharmacy, University of Technology Sydney, Sydney, NSW4.

Introduction. Pharmacists are among the most commonly sought health care providers among people with Low Back Pain (LBP) and are well positioned to provide appropriate management advice to these people.

Aims. This study aimed to investigate pharmacists’ knowledge, attitudes and beliefs towards LBP and whether participation in an educational workshop can influence these.

Methods. Pharmacists were invited to attend an evidence-based 2 h educational workshop on LBP management. Knowledge, attitudes and beliefs towards LBP were evaluated before (pre-) and after (post-) the educational workshop using the “Pharmacists’ Back Beliefs Questionnaire” (PBBQ) with items from two validated back beliefs questionnaires (Buchbinder et al, 2009; Symonds 1996). Participants indicated their agreement with statements about LBP on a 5-point Likert scale of 1 “Strongly Disagree” to 5 “Strongly Agree”. Preferred responses were based on current guidelines for the evidence-based management of LBP.

Results. Responses from 204 pharmacists participating in the educational workshop and who completed the pre- and post- PBBQ showed that the educational workshop led to significant changes to misconceptions regarding bed rest (median pre- and post- scores respectively (IQR): 3 (2-4) vs 1 (1-1) n=204; p<0.001) and the need for imaging in non-specific low back pain (median pre- and post- scores respectively: 3 (3-4) vs 1 (1-2) n=204; p<0.001).

Discussion. The provision of an educational workshop on the evidence-based management of LBP can significantly influence pharmacists’ knowledge, attitudes and beliefs towards LBP so that it more closely aligns with current evidence-based guidelines. Given the positive results, the next challenge is to develop strategies to reach a larger number of pharmacists and to expand such interventions to allied health care providers.


Online support for pharmacology practical teaching
David Dewhurst & Ross Ward. Learning Technology Section, College of Medicine & Veterinary Medicine, University of Edinburgh, UK. (introduced by Elizabeth Davis, Monash University, Victoria).

Introduction. Typical UK BSc Pharmacology courses now deliver far fewer hours of practical teaching than a decade ago. Many university departments have, in part, replaced practical classes with computer simulations developed by third party organisations and thus have little control of the content of those simulations or what is being delivered to their students.

Aim. To develop a database of quality-assured traces from a variety of tissue/whole animal preparations typically used in undergraduate education which teachers can access, download and incorporate into their own teaching materials. Other resources such as textual descriptions, animations and video-recordings will also be made available together with a laboratory manual in the form of an e-book.

Methods: Design and build an online searchable repository and populate with quality-assured resources. Develop a laboratory manual in e-book format describing a range of exemplar student activities which can be developed using the resources available in the database.

Results: the structure and functionality of the database will be demonstrated and exemplar sections of the e-book will be described. As a result of this development pharmacology students will have access to accurate data from experiments investigating the effects of various drugs/drug combinations on a number of in vitro and in vivo pharmacological preparations (the start point will be the >1000 traces currently owned by the author). The development will support research-informed student learning by providing students with access to primary data sources, via teacher designed learning activities, which will support acquisition of knowledge and a variety of skills such as data handling (measurement, presentation, interpretation), experimental design and communication. The online laboratory manual will provide exemplars of learning activities.

Discussion. Making resources of this type freely available and accessible to teachers of pharmacology will benefit student learning by supporting a variety small group teaching sessions, lectures and independent learning activities.
The development of an experiential and learning programme for undergraduate pharmacy students at Alcohol and Drug Services

Juanita L Westbury¹, Kathryn Law², Catherine Spiller³, Donnamay Brown⁴. Unit for Medication Outcomes⁵, Research and Education, School of Pharmacy, University of Tasmania, Hobart, TASMANIA; Alcohol & Drugs Service⁶, St John’s Park, New Town, Hobart, TASMANIA.

Introduction. Community pharmacists offer pharmacotherapy programs and are expected to detect, monitor and advise patients with substance abuse disorders. However, undergraduate students have only limited awareness of these disorders and their management by the time they graduate.

Aims. To develop, institute and evaluate a problem-based drug and alcohol educational program for students, the main component of which involves a 3-hr experiential placement at the Alcohol and Drug Service.

Methods. The alcohol and substance abuse educational and experiential program consisted of three lectures and a three-hour placement for 72 third year students. To assess impact the brief Substance Abuse Attitude Survey (BSAAS) and an alcohol and drugs knowledge-based questionnaire was completed by all participants. The difference between the pre and post scores was statistically compared. Students were also asked to complete a qualitative questionnaire about the placement.

Results. A total of 62 students completed the baseline surveys, with 42 of these surveys matched up to post-surveys, giving an overall response rate of 58%. Seventy-seven percent of respondents were female, with ages ranging from 20-36 years (M = 22.80, SD = 3.24). No significant differences were found between the pre and post BSAAS. However, the knowledge-based questionnaire had a significant increase in correct answers, (p<0.005), and decrease in ‘don’t know’ responses, (p< 0.005). Forty-eight students (66%) completed the qualitative questionnaire. Sixty-three percent said that their attitude towards people with substance abuse issues had changed as a result of the placement. Common themes were that students attained greater appreciation of the challenge of fighting substance abuse and that negative stigma was unhelpful. All students recommended the placement for future pharmacy undergraduates.

Discussion. Despite limited quantitative evidence of increasing positive attitudes, students’ knowledge about substance abuse significantly increased as of the program. Qualitative analysis suggests that the placement should be continued in future.

An international survey of health literacy education within schools of pharmacy

Glen Swinburne¹, Lynne Emmerton², Jeff Hughes², Peteris Darzins¹, Kay Stewart¹, Therese Kairuz³, Remo Ostini³, Betty Chaar⁴, Kylie Williams⁵, Safeera Hussainy¹, Kevin McNamara⁶,⁷, Kreshnik Hoti⁵, Robert Bush⁶, Fran Boyle¹, Moyez Jiwa³, Bill Suen⁶, Gregory Duncan¹. Centre for Medicine Use and Safety, Monash Univ¹, Melbourne, VIC; School of Pharmacy, Curtin Univ², Perth, WA; School of Pharmacy, The Univ of Queensland³, Brisbane, QLD; Faculty of Pharmacy, The Univ of Sydney⁴, Sydney, NSW; School of Pharmacy, Univ of Technology Sydney⁵, Sydney, NSW; Pharmaceutical Society of Australia⁶, Melbourne, VIC; Flinders Univ and Deakin Univ⁷, Warrnambool, VIC.

Introduction. Health literacy is defined as the ability to access, understand, appraise and communicate health information. Initiatives to improve doctors’ awareness of health literacy challenges have been reported internationally, but the concept is relatively new to pharmacy. The incorporation of health literacy in academic pharmacy curricula has not been examined. Taking stock of current health literacy training will help to develop an understanding of the needs of the pharmacy profession in health literacy.

Aims. To examine methods for teaching health literacy in schools of pharmacy internationally.

Methods. An anonymous online questionnaire was developed to examine health literacy education in pharmacy curricula, with reference to key themes identified by our group’s literature research. These included how health literacy is defined, if and when in a course it is taught, the expertise of those teaching it, resources used to enhance teaching, and perspectives on the importance of teaching health literacy. We targeted academics who taught within pharmacy degree courses from countries where English is the main language, identified through academic networks.

Results. Twenty-three academics participated from 21 schools of pharmacy in seven countries. Of these, 21 stated that health literacy was taught within their pharmacy degree, in four as a stand-alone topic. Drivers were predominantly professional practice standards and the scope of pharmacy practice in their country. The majority of respondents (16) stated that health literacy was taught later in the degree (third or fourth year). Small-group tutorials and lectures were the most commonly reported forms of teaching.

Discussion. Of our limited sample, the majority of schools of pharmacy reported teaching health literacy using a variety of teaching methods. The results will inform the content and structure for a health literacy educational package for pharmacists and pharmacy assistants in Australia that could be adopted internationally.
Experimental Design and Statistical Analysis in Intermediate Medical Sciences Curricula – A Pilot Study
Tina Hinton1, Miriam Frommer2, Vanessa Gysbers3. School of Medical Sciences (Pharmacology), Univ of Sydney1, NSW; School of Medical Sciences (Physiology), Univ of Sydney2, NSW; School of Molecular Biosciences, Univ of Sydney3, NSW.

Introduction. Graduates of pharmacology and other medical science disciplines are expected to have sufficient skills to understand experimental design, collect and analyse data, and draw conclusions from experimental results. To achieve these graduate outcomes, students must learn the tools for quantitative literacy and research enquiry in science. However there is often a gap between when students learn experimental design and statistical analysis (EDSA) - often in junior units of study - and when they use them later in their studies, work or research (Gordon and Nicholas, 2010).

Aims. To develop, implement and evaluate a pilot module for EDSA, integrated in a discipline-specific context in an intermediate level medical science curriculum.

Methods. Pharmacology and physiology unit coordinators were surveyed on their expectations of graduates in EDSA skills. Responses were used to help develop two lectures and two tutorials which were delivered across semesters 1 and 2, to convey the basics of EDSA, along with discipline-specific experimental scenarios and data. A brief survey of student confidence in EDSA was conducted in semester 1, and a formative quiz (7 questions) to assess statistical understanding before and after the lecture and tutorial was carried out in semester 2.

Results. Seven learning modules and two case studies were developed. The themes of the modules are: understanding medication safety; types and causes of medication errors; wrong drug errors; wrong route errors; intravenous errors, formulation errors and communication. The modules aim to ensure participants understand why errors occur, how to avert them in their own workplace and what systems are in place to assist safe medication management. The modules include activities and real examples of errors to engage the user and encourage reflection. The interactive case studies illustrate the safety points made in the modules. Users are provided with a certificate at completion of each module and educators are able to monitor students’ progress.

Discussion. An online medication safety training course has been developed that provides a broad overview of safety issues for all professionals involved in medicines management in acute care. The interdependency of healthcare professionals when managing medicines is a key message. The next steps regarding the use of this educational tool include widespread implementation across Australian healthcare, evaluation of the tool and, upon proven success, development of further modules and case studies.

Does evidence-based education on complementary medicines change students’ attitudes and likelihood of recommending them in a pharmacy setting?

Liesl Blott, Jeff Hughes. School of Pharmacy, Curtin Univ, Perth, WA

Introduction. Curtin University introduced a core evidence-based complementary medicine (CM) unit into the 3rd year pharmacy syllabus in 2010, consistent with Australian Pharmacy Council accreditation standards (2009) for pharmacy curriculum content. The unit was designed to provide students with the knowledge and ability to incorporate CMs into their pharmacy practice, thus meeting increased consumer demands for CMs and in keeping with pharmacists’ professional responsibilities.

Aims. Research was conducted to evaluate the impact of the evidence-based CM unit.

Methods. Pre- and post-unit surveys were administered over two consecutive years to assess changes in students’ attitudes, knowledge and likelihood of recommending CMs following evidence-based CM education.

Results. CM education resulted in a positive change in pharmacy students’ perceptions towards CMs and increased their willingness towards recommending CMs within a pharmacy setting. Survey results show that completion of the unit led to a statistically significant positive change towards a belief that there is evidence for a number of CMs for the treatment of several conditions, diseases or for symptom management [2010, \( p=0.002 \); 2011, \( p=0.027 \)] and that there is scientific evidence to support efficacy of many CMs, beyond a placebo effect [2010, \( p=0.002 \)]. There was also a statistically significant shift in students’ personal confidence in making recommendations to customers as part of a pharmaceutical care plan [2010, \( p=0.000 \); 2011; \( p=0.000 \)].

Discussion. The evidence-based CM unit at Curtin University proved valuable in equipping pharmacy students with the ability and willingness to make appropriate patient-specific CM recommendations as part of their professional pharmacy practice, taking evidence on efficacy, safety and place in therapy into consideration, and in line with pharmaceutical care educational guidelines for pharmacy students. This educational initiative is a valuable step forward in bridging the gap between the identified need for greater evidence-based CM instruction and the reality of current pharmacy educational practices.

---

Use of an audience response system and collaborative learning increases engagement in post graduate pharmacy.

Slade Matthews, Ian Spence and Peter Carroll. Pharmacology, Sydney Medical School, Univ of Sydney, NSW

Introduction. Over the last few years one of the most successful implementations of technology in the learning environment has been the use of audience response systems (Cain, Black, & Rohr, 2009). These systems are shown to increase student engagement in a variety of learning environments. In my post graduate pharmacy course I detected a need for an intervention with the ability to engage students while listening to peer presentations.

Aims. I aimed to increase the benefit students derived from student presentations in terms of student engagement.

Methods. A series of clinical-style cases were devised as motivational examples to increase immediacy and highlight the relevance of the material to students. Student groups were required to present a case in turn each week. Each student within a group had to set an MCQ on an important point from the case. The whole class was tested at the end of each presentation using an audience response system (Votapedia, www.urvoting.com).

Results. Student participation (quiz responses) averaged 72% of the class over semester (range 53 – 90%, SD = 12%). In structured interviews students revealed they found setting questions with Votapedia increased the meaning they attributed to their presentation and that collaborative learning provided opportunities for increasing their own understanding.

Discussion. The participation rate and student feedback indicate how students valued the activity. This strategy provides an opportunity to demonstrate the benefit of collaborative learning as well as the use of technology to increase student engagement with the curriculum.

Exploring community pharmacists’ views on medication-safety management
Rachel A George¹, Timothy F Chen¹, Andrew J McLachlan¹,², Romano A Fois¹. Faculty of Pharmacy, Univ of Sydney¹; CERA, Concord RG Hospital, NSW²

Introduction: Preventable harm from medicine use in the community continues to occur despite the best intentions of healthcare professionals and strategies to support quality use of medicines. Incident Reporting Programs (IRPs) can identify system weaknesses and generate strategies to prevent medication-safety incident (MSI) recurrence. IRPs depend on healthcare professional engagement. An IRP was offered to pharmacists alongside a medication-safety campaign in 2011; however, few pharmacists engaged. This may stem from pharmacists’ perceptions of IRPs and the profession’s role in reporting. Beliefs of pharmacists towards current MSI management have not been explored.

Aims: To gain insight into (1) community pharmacists’ perspectives on management and response to MSIs and (2) barriers and facilitators to IRP participation.

Methods: Semi-structured interviews involved presentation of 1-2 MSI vignettes followed by discussion of contributing factors. Workplace responses to these were explored. The interviewer provided education on how system failures contribute to MSIs and the potential for IRPs to inform systems change, followed by reflection on beliefs of MSI management and the role of IRPs. Barriers and facilitators to IRP participation were discussed.

Results: Interim analysis of 11 interviews reveals blame culture as a factor in typical workplace responses. Lack of time, competing priorities and fear of punishment for MSI disclosure were reported as barriers to IRP participation. Facilitators included education on the systems approach to MSIs, as well as incentives (e.g. continuing professional development points).

Discussion: Our findings suggest pharmacists possess a broad range of beliefs and approaches towards MSI management depending on individual workplace culture. Future medication-safety initiatives will need to consider pharmacists’ beliefs and needs, including education and additional resources and incentives for reporting.

Safety before efficacy? Australian pharmacists’ attitude to homeopathic products in pharmacy
Suzanne J Schultz¹, Elizabeth D Hotham¹, Allan M Evans². School of Pharmacy and Medical Sciences, University of South Australia¹, Adelaide, SA; Division of Health Sciences, University of South Australia², Adelaide, SA.

Introduction. Despite Cochrane reviews concluding there is no evidence for the efficacy of homeopathic preparations, they are sold in most community pharmacies in Australia. This availability may put professional credibility at risk.

Aims. This study explored factors that influence pharmacists’ preparedness to recommend, or at least make available, homeopathic products.

Methods. A mixed method approach was used incorporating two focus groups of South Australian pharmacists with a variety of backgrounds (n=13); telephone interviews with pharmacists from each state and territory (n=18), and a cross-sectional survey for community pharmacists (n=185). Non-parametric tests were used to identify significant patterns in attitudes toward OTC and homeopathic products.

Results. More pharmacists ranked efficacy above safety in OTC products and were more likely to discourage homeopathic product use, than those who ranked safety before efficacy. Almost all (96%) indicated that they would like the public and health colleagues to think of them as a source of information about all medicine and health products. Pharmacists believe that they will only sell a product if it will be useful for a patient, with homeopathic products sometimes regarded as a “safe” option for consumers. Some pharmacists see a place for these products if consumers seek them, although few would personally recommend them. Those who did not support their provision were concerned with the reputation of the profession. The impact of homeopathic products on business profitability was found to be minimal.

Discussion. Pharmacists want to be seen as caring for the health of consumers and as medication experts. Pharmacists want access to reliable information sources, education, and desired that undergraduate education should include skills to critically evaluate evidence for a range of health products. There is a strong argument for removing homeopathic products from pharmacy based on the lack of evidence, lack of commercial return and risk to credibility.
Improving consumer access to medicines: innovative medicines reclassification in New Zealand (NZ)
Natalie Gauld1, Lynne Emmerton2, Fiona Kelly3, Stephen Buetow1. Department of General Practice and Primary Healthcare, University of Auckland1, Auckland, NZ; School of Pharmacy, Curtin University2, WA; School of Pharmacy, University of Auckland3, Auckland, NZ.

Introduction. Prescription to non-prescription medicines reclassification is generally regarded as a positive development to increase consumer access to medicines and relieve limited health resources, although reclassification rates have slowed in many developed countries. Despite a small population and negative pharmaceutical industry environment, reclassification is progressing in NZ. Recent changes include influenza vaccination, and first-in-world reclassifications such as calcipotriol and trimethoprim.

Aims. To ascertain why NZ is one of the most progressive developed countries regarding medicines reclassification.

Methods. Two main sources of data were used. Interviews were conducted with 12 purposively selected key informants on NZ medicines reclassification, exploring barriers and enablers for reclassification. Secondly, analysis of minutes of the Medicines Classification Committee (1990-2011), and related documents provided reclassification rates and insight into deliberations around each case. The experience of the first author in reclassification in NZ was also used. A heuristic approach was used to thematically analyse the content of the transcribed interviews, which were then compared with 54 similar interviews from other countries including Australia, the United Kingdom, the United States and Japan.

Results. Multiple enablers to reclassification exist in NZ, with the key themes arising from the interviews including: the pharmacist-only medicine category; a ‘can do’ approach; the smallness of the NZ market; openness to change; trust in pharmacy and consumers; harmonisation with Australia; and facilitation by key individuals. Mandatory training, and non-sponsor reclassification (by a pharmacy retail group) enable reclassification. The pharmacy retail group has driven four reclassifications, with another pending.

Discussion. Innovation, as has occurred in NZ, may provide useful insights to move reclassification forward internationally. Research is required to ascertain the effect of non-prescription provision of these medicines on patient outcomes.

Community-acquired pneumonia: why aren’t national antibiotic guidelines followed?
Maher Almatar1, Gregory Peterson1, and Duncan McKenzie2, Tara Anderson2 School of Pharmacy, University of Tasmania1, Royal Hobart Hospital2, Hobart, Tasmania, Australia

Aims: To assess adherence to the Australian Therapeutic Guidelines (TG14) for the empirical management of community acquired pneumonia (CAP), and explore the potential barriers affecting adherence to these guidelines.

Methods: Medical records were reviewed for all patients who were diagnosed with pneumonia within 24 hours of presentation (either admitted or not) at the Royal Hobart Hospital (RHH) from June 2010 to March 2011. A survey to identify potential barriers affecting adherence with TG14 was distributed to prescribers in the RHH’s emergency department and medical units.

Main measuring outcomes: The adherence rate to TG14 for the management of CAP in terms of selected antibiotic, route of administration and dose; and the extent of doctors’ agreement with the statements in the survey regarding the potential barriers.

Results: A total of 193 patients were assessed. The overall adherence to TG14 for the empirical antibiotic management of CAP was 23.8% (25.5% and 22.6% for patients with severe and non-severe CAP, respectively). Twenty-nine different antibiotic regimens was utilised during the audit period. Ceftriaxone-based therapy was prescribed to 57% and 30% of patients with severe and non-severe CAP, respectively. The response rate to the barriers survey was 50.9%; of those who responded 46.4% thought the influence of senior doctors could be a factor affecting junior doctors’ adherence to the guidelines. Other barriers noted were a lack of guideline awareness (39.3%), the requirement to calculate to assess the severity of CAP (35.7%), and the existence of other guidelines that conflict with TG14 (28.6%).

Conclusion: Adherence with TG14 was poor for the treatment of CAP. Efforts to improve this should consider the potential barriers that hinder adherence.
Implications of the Personally-Controlled Electronic Health Record for community pharmacy
Lynne M Emmerton¹, Armin Mooranian¹ & H Laetitia Hattingh¹. School of Pharmacy, Curtin Health Innovation Research Institute, Curtin Univ¹, Perth, WA.

Introduction. International developments embrace the concept of electronic health records for consumers, aiming to reduce fragmentation in health data. The Australian Government has driven the national implementation of Personally-Controlled Electronic Health Records (PCEHRs) from 1st July 2012. Despite national and international data addressing the impact of e-health records on logistical and professional aspects of practice from doctors’ and nurses’ perspectives, data regarding the impact on community pharmacy is almost non-existent.

Aims. To determine, in the months prior to the launch, community pharmacists’ perceptions about the developments and how they might integrate PCEHRs into pharmacy practice.

Methods. Semi-structured interviews of 20-25 minutes’ duration were undertaken during March-April 2012 with 25 pharmacy owners and managers from 24 community pharmacies in Perth, Western Australia. Independently-owned and ‘banner group’ pharmacies were included from a range of suburbs. Interviewees were briefed about the PCEHR, before exploratory questions regarding the potential integration, benefits and challenges of the system in pharmacy practice. Data were recorded, transcribed and thematically analysed.

Results. Most pharmacists perceived benefits in enhanced access to patient data, and expected system flexibility to record clinical activities and health services. Patients’ control over their data management was a concern, potentially resulting in incomplete and untrustworthy data, with potential for litigation of health professionals for decisions made on incomplete data. Concerns were also raised about workload, technical upgrades and work flow. The pharmacists called for remuneration, medico-legal guidelines and boundaries, and clarification of roles and responsibilities. The pharmacists universally voiced a need for PCEHR training.

Discussion. Awareness of the perceived benefits and challenges with the PCEHR will advise Australian practice guidelines, training priorities and policies to assist with adoption and optimal use of PCEHRs by community pharmacists. Training priorities and practice guidelines should address ethical data management and optimal use of electronic health records for clinical services.

Challenges and safety concerns for community pharmacy personnel in the provision of services to young people
Emma Horsfield¹, Janie Sheridan¹, Fiona Kelly¹. School of Pharmacy, University of Auckland¹, Auckland, NZ

Introduction. Young people aged 12-25 are a population with specific health needs distinct from those of either children or adults. Research indicates that these additional considerations can present challenges to primary healthcare providers. This study explored provision of services to youth from the perspective of community pharmacy personnel.

Aims. To explore challenges faced by community pharmacists and pharmacy staff in the provision of services to young people.

Methods. Questionnaires were distributed to pharmacy personnel at 500 randomly selected pharmacies across New Zealand. In addition to quantitative data collected from youth-health related questionnaire items, an open question section collected qualitative data on i) participants’ comments and experiences, ii) participants’ suggestions for development of services for youth. These data were analysed in NVivo using a general inductive approach to identify barriers, facilitators, suggestions and training needs. This presentation will describe themes relating to safety concerns and challenges.

Results. Three mail shots yielded response rates of 50.5% for pharmacists and 37.1% for pharmacy staff. Just over a third of participants (n = 171) answered the open questions. Common concerns relating to safety included beliefs that young people are less health literate, and seek information from unreliable sources such as peers or the internet. Young people were also reported to be less adherent, and more likely to require referral. Participants were concerned about broad safety and wellbeing issues for young people, particularly with regards to provision of sexual health services, and medicines with potential for misuse such as weight management products.

Discussion. Many challenges faced by pharmacists and pharmacy staff providing services to young people relate to concerns about the safety or appropriateness of medications and services for this vulnerable population. Clarification of guidelines and legislation regarding this age group is necessary to support pharmacy personnel and improve quality of care for youth.
**End-users’ perceptions of the electronic medication repository, MedView.**

Carl MJ Kirkpatrick¹, Diana Bortoletto¹,², David CM Kong¹, Robert Franken³ & Safeera Y Hussainy¹, Centre for Medicine Use & Safety, Monash Univ¹, Parkville, VIC; Pharmacy Dept., Barwon Health², Geelong, VIC; Fred IT Group Pty Ltd³, Abbotsford, VIC.

Introduction. MedView, developed as part of the Australian government’s personally controlled electronic health record initiative, is an electronic medication repository that allows access to patients’ medication histories across hospital and community settings. It was trialled in early 2012 in the Barwon region, Victoria.

Aims. To evaluate end-users’ perceptions of MedView at “Pre-” and “Post-” implementation.

Methods. “Pre-MedView” evaluation involved semi-structured interviews and surveys with hospital doctors, general practitioners, and hospital and community pharmacists. “Post-MedView” implementation involved semi-structured interviews of healthcare practitioners and consumers. Interviews were transcribed verbatim and thematically analysed. Data was triangulated.

Results. “Pre-MedView”: Health professionals (n = 38) were interviewed, and 14% (123/875) of surveys were returned. General practitioners (78%), hospital doctors (80%), hospital pharmacists (74%) and community pharmacists (52%) had difficulties accessing accurate medication information at least some of the time in current practice. MedView’s perceived benefits included facilitating continuity of care between different healthcare settings, reducing medication misadventure and improving work efficiencies. The opt-in consent process in community was thought to negatively influence work efficiencies. Hospital pharmacists were likely to be the most frequent MedView users (58% likely to use for ≥ 60% of all patients). Perceived barriers included incomplete medication information in MedView. Concerns about possible misinterpretation of information and increase rate of medication errors were raised. “Post-MedView”: Health professionals (n = 24) and 38 consumers participated. Most viewed MedView as user-friendly and easily accessible. Most consumers (84%) consented to sharing their medication information on MedView.

Discussion. MedView was perceived to have the potential to provide benefits, in terms of improving medication use and safety across the continuum of care. MedView was user-friendly. Refinement in roll-out and education strategies will be required for wider roll-out.

**Management of high blood pressure in pregnant women attending an Australian maternity hospital**

Amyna Helou¹, Kay Stewart¹, Johnson George¹; Centre for Medicine Use and Safety, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University¹, Melbourne, VICTORIA

Background: Hypertension complicates approximately 10% of pregnancies in Australia. While the importance of managing severe hypertension is undisputed, management of mild-moderate hypertension remains contentious.

Aim: To review antenatal management of women with pre-pregnancy or pregnancy-induced hypertension (PIH), and compare outcomes.

Methods: An electronic search identified women, who gave birth at Mercy Hospital for Women in 2010, with an ICD code corresponding to any hypertensive disorder of pregnancy. A manual record review of eligible patients was performed to compare pregnancy and blood pressure(BP)management, perinatal and obstetric outcomes according to diagnosis of PIH and pre-pregnancy hypertension, and between women receiving treatment and their untreated counterparts.

Results: 513 women (9.1%) were identified as having hypertension – pre-pregnancy hypertension (n=59, Group 1) or PIH (n=454). Among women with PIH, 76 (16.7%) received treatment (Group 2) and 378 did not (Group 3). The women in Group 1 were significantly older than those in Group 3 (p < 0.001). Group 2 had the most frequent development of pre-eclampsia (any form)(64%), shortest gestation (35 weeks and 2 days± 4 weeks and 3 days) and highest number of babies with fetal growth restriction(26%) in comparison with the other two groups; one baby in this group was stillborn. Group 3 had the only two incidences of placental abruption and one separate incidence of fetal death. The BP reading at which hypertension was diagnosed in Group 2 was significantly higher than in Group 3 (152/94mmHg vs. 142/90mmHg;p=0.001). The diagnosis of hypertension was also significantly earlier in Group 2 (31 weeks and 1 day± 4 weeks and 4 days vs. 35 weeks and 2 days ± 4 weeks;p=0.001)

Conclusion: The majority of women who develop PIH are managed by close monitoring without antihypertensive medication. There is potential for improvement of BP management in both the untreated and treated groups.
Pharmacovigilance and computational chemistry approaches to predicting torsade de pointes
Christabel E Abalo1, David E Hibbs1, Romano A Fois1. Faculty of Pharmacy, The University of Sydney, Sydney, NSW.

Introduction. Drug-induced QT interval prolongation has been linked to a rare, yet fatal tachyarrhythmia torsade de pointes (TdP). The majority of drugs associated with QT prolongation and TdP, including antiarrhythmics, antihistamines and antibiotics have a common pharmacologic action on potassium ion channel (hERG) inhibition. Early identification of QT liability and torsadogenic risk of drugs is a critical aspect of the drug development process.

Aims. To combine pharmacovigilance and computational chemistry methods to predict drug mediated torsadogenic risk.

Methods. Case reports of TdP were retrieved from the Australian pharmacovigilance database. A list of drugs with suspected TdP liability from literature was used as a reference to define drug searches. The reporting odds ratio as a measure of disproportionality was calculated for each drug using binary logistic regression to signal active drugs. These were used to develop a pharmacophore hypothesis and were docked into a homology model of the hERG channel.

Results. A significant disproportionality was observed for amiodarone, sotalol, erythromycin, azithromycin, linezolid, thioridazine, citalopram, cisapride, methadone, terfenadine, flucloxacillin, ribavirin, zidovudine and levosimendan. The generated pharmacophore model contained three hydrogen bond acceptors and one aromatic ring feature. Docking of the drugs into the homology model revealed that amino acids THR623, SER624, SER649, TYR652, and PHE656 were consistently involved in interactions with the ligands, consistent with the spatial distribution of the pharmacophoric features.

Discussion. Amino acids identified to interact with ligands particularly TYR652 and PHE656 are consistent with alanine-scanning mutagenesis which have identified TYR652 and PHE656 as crucial for hERG blockade by cisapride and terfenadine. Pharmacovigilance databases contain a wealth of information which can be utilised to guide in silico methods of predicting torsadogenic risk.

Tocomin® restores endothelium-dependent relaxation in diabetic rat aorta.
Saher F Ali & Owen L Woodman. School of Medical Sciences, Health Innovations Research Institute RMIT University, Bundoora, VIC.

Introduction: Tocotrienols, a component of vitamin E with structural similarities to tocopherols, may have beneficial effects on the vascular function particularly in pathologies involving oxidant stress such as diabetes. Tocomin® is an extract of palm oil with a high tocotrienol content.

Aims: To determine the effect of tocotrienol rich tocomin® (composition: tocotrienol rich fraction: 40%, α-tocopherol: 11% and palm olein: 38%) treatment on endothelium-dependent and –independent relaxation in the diabetic rat aorta.

Methods: Male wistar rats were randomly assigned to 4 groups (control, control+tocomin®, diabetic and diabetic+tocomin®). Diabetes was induced by a single injection of streptozotocin (50 mg/kg iv). Rats were by treated with tocomin® (40 mg/kg per day s.c.) or vehicle (100% peanut oil) for a period of 4 weeks commencing 6 weeks after induction of diabetes. Acetylcholine (Ach)-induced endothelium-dependent and sodium nitroprusside (SNP)-induced endothelium-independent relaxation was measured in rat aortae using standard organ bath techniques.

Results: STZ increased blood glucose (control, 7.1 ± 0.4 mmol/L, STZ 29 ± 2.3 mmol/L, n=15-17) and glycated haemoglobin (HbA1c control, 5.5 ± 0.5%, STZ 12.5% ± 2.8%, n=15-17). Neither parameter was affected by tocomin® treatment in diabetic rats (BGL control+tocomin®, 7.3 ± 0.7 mmol/L, diabetic+tocomin® 26.3±4 mmol/L, HbA1c control+tocomin® 5.5±0.2%, diabetic+tocomin® 11.8±1.3%, n=8-10). Diabetes impaired Ach induced endothelium-dependent relaxation (Rmax control 95±2% vs diabetic 82±4%, n=6-8, p<0.01, pEC50 7.50 ± 0.23 vs 6.6 ± 0.13 diabetic, n=6-8, p<0.01) without affecting SNP-induced relaxation. Tocomin® treatment significantly improved endothelium-dependent relaxation (pEC50 diabetic+tocomin®, 7.2 ± 0.13 n=6-8, p<0.05).

Discussion: These findings demonstrate that 4-week treatment of diabetic rats with tocotrienol rich tocomin® significantly improves endothelium-dependent relaxation in diabetic rat aorta without affecting blood glucose levels.
Head to head comparison of the relative anti-fibrotic efficacy of H2 relaxin to a clinically used ACE inhibitor (Enalapril)

Hasangika K Bodaragama, Simon G Royce & Chrishan S Samuel. Fibrosis Laboratory, Department of Pharmacology, Monash University, Clayton, VIC

Introduction: Organ scarring (excess matrix/collagen accumulation) is the final endpoint of numerous cardiovascular diseases, for which there is currently no effective cure.

Aims: This study aimed to compare a novel anti-fibrotic (relaxin) to that of a clinically used angiotensin converting enzyme inhibitor (ACEi; enalapril) in a mouse model of isoproterenol (ISO)-induced ischemic heart disease/fibrosis.

Methods and Results: Repeated s.c administration of isoprenaline hydrochloride (ISO) for 5 consecutive days induced a 2-fold increase in aberrant collagen deposition and 11-12-fold increase in picrosirus red-stained interstitial collagen, 12 days later (day 17) (both P<0.001 vs untreated controls). Increasing doses of relaxin (0.5, 1, 2mg/kg/day) and enalapril (200, 300, 500mg/L) (n=5-6 mice/treatment group), delivered via osmotic mini-pumps and drinking water, respectively, were first evaluated for their ability to prevent ISO-induced collagen levels. All doses of relaxin tested significantly prevented total collagen concentration (hydroxyproline assay) and picrosirus-red stained interstitial collagen (morphometry) by 55-60% (all P<0.01 vs ISO alone), without affecting blood pressure (tail-cuff). The lowest dose of enalapril (200mg/L) also prevented ISO-induced collagen levels by about 50% and this response was delayed by 150 minutes when ERK1/2 was inhibited but was unaffected by inhibition of PI 3-Kinase.

Discussion: These findings indicate that relaxin increases renal sympathetic nerve activity via PI 3-kinase but reduces BAT SNA via ERK1/2. Since the plasma levels of resistin are elevated in obesity, resistin may contribute to the cardiovascular and metabolic dysfunction in obesity.

Resistin, a novel adipokine, increases renal SNA and reduces BAT SNA using PI 3-kinase or ERK 1/2 mediated mechanisms

Emilio Badoer¹, Joseph Rathner² and Samin Kosari¹. School of Medical Sciences, RMIT University¹, Human Bioscience, Latrobe University², Melbourne, Victoria

Introduction. A characteristic of obesity is a marked elevation of sympathetic nerve activity (SNA) to the skeletal muscle blood vessels and to the kidney which contribute to obesity induced hypertension. The causes of the increase in SNA are not known. Adipose tissue is now recognised as a major endocrine organ that releases many hormones, including leptin, adiponectin and, more recently, resistin. Leptin and adiponectin have cardiovascular and metabolic effects that involve actions in the central nervous system that influence SNA. Leptin, for example, increases SNA to the kidney and brown adipose tissue (BAT), a metabolic organ, but little is known of the effects of resistin.

Aims. To investigate the effect of centrally administered resistin (i) on SNA targeting the kidney and BAT, and (ii) the intracellular signalling pathways mediating those changes.

Methods. Rats were anaesthetised (urethane 1.4 g/kg iv) and renal or BAT SNA was recorded using standard methodology. Resistin (7ug) was injected into the lateral cerebral ventricles (icv) in the presence or absence of inhibitors of the enzymes P I3-Kinase (LY294002, 5ug) or ERK 1/2 (U0126, 7ug). The changes in SNA, blood pressure and heart rate were monitored and compared to control groups.

Results. Resistin induced a significant increase in renal SNA by approximately 40%. This response was prevented when PI 3-Kinase was inhibited but was unaffected by ERK 1/2 inhibition. In contrast, resistin reduced BAT SNA by about 50% and this response was delayed by 150 minutes when ERK1/2 was inhibited but was unaffected by inhibition of PI 3-Kinase.

Discussion. The findings indicate that resistin increases renal sympathetic nerve activity via PI 3-kinase but reduces BAT SNA via ERK1/2. Since the plasma levels of resistin are elevated in obesity, resistin may contribute to the cardiovascular and metabolic dysfunction in obesity.
Comparing the anti-fibrotic actions of relaxin versus an angiotensin AT1 receptor blocker and AT2 receptor agonist
Jacqueline Chew, Simon G Royce, Chrishan S Samuel. Fibrosis Laboratory, Dept of Pharmacology, Monash University, Clayton, VIC

Introduction: Fibrosis is a hallmark of several forms of cardiovascular disease, current front-line treatments such as angiotensin receptor blockers (ARBs) only modestly prevent its progression.
Aims: This study aimed to compare the efficacy of emerging anti-fibrotics (relaxin and an AT2 receptor agonist, CGP42112) against a clinically used ARB (candesartan cilexetil), in a mouse model of isoproterenol (ISO)-induced ischemic heart disease/cardiac fibrosis (Brooks and Conrad, 2009).

Methods and Results: Hydroxyproline analysis (total collagen concentration) and morphometry (picrosirius red-stained interstitial collagen) showed that increasing doses of relaxin (0.5, 1, 2mg/kg/day, via mini-pumps) similarly prevented ISO-induced fibrosis progression (by 55-60% vs ISO alone, P<0.001); and to a greater extent than candesartan (0.5, 2, 5mg/kg/day via drinking water) or CGP42112 (1.44mg/kg/day via mini-pumps) (n=5-6 animals/treatment group), neither of which significantly affected ISO-induced collagen deposition. Unexpectedly, combination of the optimal dose of relaxin (0.5mg/kg/day) and candesartan (0.5mg/kg/day; which had no effect on blood pressure) inhibited the anti-fibrotic actions of relaxin (on aberrant TGF-1, Smad2 phosphorylation (pSmad2) and collagen concentration) back to ISO alone-induced levels. On the other hand, combination of CGP42112 (1.44mg/kg/day) and relaxin (0.5mg/kg/day) equivalently prevented fibrosis to that of relaxin alone (P<0.01 vs ISO alone), without inducing any additive effects over either treatment alone. The enhanced anti-fibrotic efficacy of relaxin over candesartan and CGP42112 was associated with its ability to more effectively reduce TGF-1 expression/staining (P<0.05 vs candesartan or CGP42112 treatment alone). However relaxin did not demonstrate any significantly improved effects on pSmad2 (immunohistochemistry and morphometry) or on matrix-degrading metalloproteinases: MMP-13 (immunohistochemistry) and MMP-2 (gelatin zymography).
Discussion: Although further studies are required to assess the anti-fibrotic efficacy of these treatments in other models/strains of injury/disease, these findings suggest that relaxin is a more effective anti-fibrotic compared to candesartan or CGP42112 in a model of ischemic heart disease.


Is insulin-mediated sensitization of platelets to nitric oxide in diabetics thioredoxin-interacting protein-dependent?
Cher-Rin Chong1,2, Saifei Liu1,2, Nathan Procter1,2, Chloe Zhang1,2, John Licari1,2, Yuliy Chirkov1,2, John Horowitz1,2.
1Dept of Cardiology and Clinical Pharmacology, Basil Hetzel Institute, the Queen Elizabeth Hospital, SA
2University of Adelaide, SA

Introduction. Acute coronary syndrome (ACS) in diabetics is associated with substantial mortality, which is reduced by correction of hyperglycaemia 1. We have previously shown that rapid reversal of hyperglycaemia with IV insulin is associated with suppression of O2 release and restoration of normal platelet responsiveness to NO 2. We now wish to determine whether falls in BSL induce associated rapid (and potentially crucial) reductions in platelet expression of the hyperglycaemia-sensitive redox inducer thioredoxin-interacting protein (TxNIP).

Methods and Results: Patients were selected on the basis of concomitant ischaemia and BSL > 11.1 mmol/L 1. Investigations were performed before and after 12 hours of insulin infusion. These included:- platelet responsiveness to NO (whole blood aggregometry), whole blood O2 content (EPR), platelet TxNIP expression (immunostaining) and PMA stimulated leukocyte O2 release (EPR).

Results. Results of pilot study (n=4) are reported. BSL fell by 32 ± 22 (SD) % over 12 hours. This was associated with an increase in platelet NO response from 14 ± 14 (SD) to 52 ± 26 % (p<0.05), and a decrease in whole blood O2 content by 25 ± 17 %. However, there was no consistent change in neutrophil O2 release (δ = 10 ± 50 %), nor in platelet TxNIP content (δ = 72 ± 120 %).

Conclusion. While these pilot data confirm the previous findings that treatment of hyperglycaemia with IV insulin reduces O2 content and potentiates platelet response to NO, the PMA and TxNIP data do not provide insights into the mechanism(s) underlying the changes in O2 generation. It is possible that despite the glucose-responsive element in genes for TxNIP expressions modulation of such changes in (non-nucleated) platelets is inherently slow.

Utilisation of antithrombotics for secondary stroke prevention at hospital discharge
Ashraf Eissa¹, Ines Krass ¹, Beata Bajorek²,3
¹Faculty of Pharmacy, University of Sydney, Sydney, NSW; ²School of Pharmacy, University of Technology Sydney, ³Royal North Shore Hospital, Sydney, NSW.

Introduction. Stroke is a leading cause of death and disability yet it is both preventable and treatable. Significant proportions of stroke presentations are by patients with a previous stroke. For this reason, the clinical guidelines for stroke management recommend an appropriate antithrombotic drug therapy (either anticoagulant or antiplatelet) for the secondary prevention of stroke.

Aims. To determine the rates of utilisation of antithrombotic drug therapy for stroke patients and to identify factors associated with use of treatment at discharge.

Methods. A retrospective clinical audit was conducted in five metropolitan hospitals in NSW, comprising two tertiary referral centres and three district hospitals. Patients discharged with a principal diagnosis of ischaemic stroke during a 12-month time period (July 2009-2010) were identified and the medical records of a systematically chosen sample reviewed.

Results. In total, 521 records were reviewed (48.8% females; mean age 74.4±14 years; range 5-102). Overall, 97.6% of eligible patients were prescribed an antithrombotic at discharge; of these 68.5% were prescribed monotherapy. The univariate analysis identified hypercholesterolemia as the only variable associated with the utilisation of any antithrombotic therapy at discharge. For patients with atrial fibrillation (AF), 97.0% were prescribed an antithrombotic at discharge, however, only 57.4% were prescribed an anticoagulant. Multivariate logistic regression analysis identified male gender, heart disease, discharge destination (home) and functional status at discharge (independent) as significant predictors for the utilisation of anticoagulants in AF patients. As for non-AF patients 95.2% were prescribed an antithrombotic at discharge, of whom 87.9% were prescribed an antiplatelet agent.

Discussion. The majority of stroke patients received an antithrombotic at discharge. However, the utilisation of anticoagulant therapy for the secondary prevention of stroke in patients with AF is suboptimal. Additional efforts are needed to increase the utilisation of evidence-based, guideline-recommended therapies for the secondary prevention of stroke.

Blood Pressure lowering therapy for stroke patients at hospital discharge
Ashraf Eissa¹, Ines Krass ¹, Beata Bajorek²,3
¹Faculty of Pharmacy, University of Sydney, Sydney, NSW; ²School of Pharmacy, University of Technology Sydney, ³Royal North Shore Hospital, Sydney, NSW.

Introduction. Elevated blood pressure (BP) is a major risk factor for recurrent stroke. Contemporary evidence shows that lowering BP, even in the absence of hypertension, significantly reduces recurrent stroke and cardiovascular events in stroke patients. Furthermore, commencement of BP lowering therapy prior to hospital discharge significantly improves rates of adherence post discharge. Therefore, the clinical guidelines for stroke management recommend that all stroke patients (normotensive and hypertensive) receive a BP lowering therapy unless contraindicated by symptomatic hypotension.

Aims. To determine the use of BP lowering therapy for stroke patients on discharge and to identify factors associated with utilisation at discharge.

Methods. A retrospective clinical audit was conducted in five metropolitan hospitals in NSW, comprising two tertiary referral centres and three district hospitals. Patients discharged with a principal diagnosis of ischaemic stroke during a 12-month time period (July 2009-2010) were identified and the medical records of a systematically chosen sample reviewed.

Results. In total, 521 records were reviewed (48.8% females; mean age 74.4±14 years; range 5-102). Overall 75.4% of eligible patients were prescribed BP lowering therapy at discharge. The majority were prescribed either monotherapy (39.7%) or dual therapy (23.8%). In addition 40 patients were prescribed triple therapy and 15 patients were prescribed 4 antihypertensive drugs or more. Univariate analysis identified 6 independent variables as having a potential impact on the utilisation of BP lowering therapy: (either anticoagulant or antiplatelet) for the secondary prevention of stroke.

Discussion. Rates of BP lowering therapy post stroke in this population are suboptimal and consistent with the literature. Additional efforts are needed to increase the utilisation of evidence-based, guideline-recommended therapies for the prevention of cardiovascular and cerebrovascular events.
Capsaicin relaxes arteries via NO-dependent and independent mechanisms.
Kirsty A Fuller1, Russ Chess-Williams1 & Peter J Johnson1. Bond University1, Robina, QLD.

Introduction. Capsaicin, the active constituent in chilli, is known to stimulate the release of sensory neuropeptides via transient receptor potential vanilloid type 1 (TRPV1) receptors. Capsaicin also relaxes blood vessels however the mechanisms responsible are not fully understood.

Aims. To test the effectiveness of activating these two receptors with classical and novel agonists to reduce neuronal death in vitro or in vivo.

Methods. Anterior descending coronary artery segments from porcine hearts were mounted in organ baths in physiological saline. Capsaicin dose responses were performed in the presence of receptor antagonists and enzyme blockers. Nitric oxide (NO) release from tissues was quantified using a nitrate/nitrite fluorometric assay.

Results. Low concentrations of capsaicin (0.01-1 μM) evoked small amplitude relaxations and release of nitric oxide into the bath effluent which were abolished by Nω-nitro-L-arginine (L-NNA) but were unaffected by indomethacin. High doses of capsaicin (10 μM-100 μM) evoked large amplitude relaxations which remained unaffected by both L-NNA and indomethacin. The purportedly selective TRPV1 antagonist capsazepine had no effect on relaxations to high concentrations of capsaicin but unexpectedly enhanced relaxations to low concentrations by 10-20% (P<0.05 cf control) and also increased NO release. Furthermore, AVE0991 was unable to improve functional outcome or reduce infarct volume following stroke in vivo.

Discussion. These results suggest that low doses of capsaicin, in the physiological range following dietary consumption of chilli, produce vasorelaxations due to endothelial-dependent release of NO which is independent of TRPV1 receptors. This may account for the antihypertensive effects reported in other studies. High doses of capsaicin relax blood vessels by a non-selective mechanism, probably due to interference with calcium influx. Capsazepine while not causing a direct relaxation of arteries, potentiates the effects of low doses of capsaicin.

Vanilloid-like agents inhibit platelet aggregation in vitro
Safa Al-Maghrabi, Murray J Adams, Kiran DK Ahuja, Dominic P Geraghty. School of Human Life Sciences, Univ of Tasmania, Launceston, TAS.

Aim. Vanilloids exert their effects primarily through activation of transient receptor potential vanilloid 1 (TRPV1). These agents inhibit platelet aggregation and may protect against the development of cardiovascular disease. The aim was to investigate the effects of a range of vanilloid-like agents on in vitro platelet aggregation.

Methods. Collagen-, ADP- and arachidonic acid (AA)-induced platelet aggregation (%Max, %AUC, slope) was determined in the absence and presence of capsaicin (CAP), dihydrocapsaicin (DHC), N-oleoyldopamine (OLDA) and N-arachidonoyl-dopamine (NADA). Lactate dehydrogenase (LDH) release was measured to determine the direct toxic effects of vanilloids on platelets. Finally, PF4 and β-TG release were measured to determine the effects of vanilloids on alpha granule release.

Results. ADP-induced aggregation was inhibited in a concentration-dependent manner by CAP (%Max, mean±SEM; 0 vs 100 μmol/L, 83.8±0.9 vs 45.2±2.4, p<0.001); OLDA (71.6±8.2 vs 9.4±1.4, p<0.001); NADA (71.5±5.9 vs 38.2±1.4, p<0.008). OLDA (89.3±1.4 vs 45.5±12.5, p<0.001) and NADA (87.7±0.8 vs 28.5±8.2, p<0.001) inhibited aggregation induced by collagen. AA-induced aggregation was inhibited by CAP (89.6±0.9 vs 11±0.8, p<0.001); DHC (88.3±2.1 vs 18.7±6.9, p<0.001); and NADA (84±1.8 vs 21.9±4.7, p<0.001). The rate of aggregation (slope) was not affected. As LDH release was not affected by vanilloids, inhibition of aggregation was not due to a direct toxic effect. The TRPV1 antagonist, SB-452533, did not affect inhibition of ADP-induced aggregation by OLDA or CAP, suggesting that inhibition of aggregation by vanilloids is not TRPV1 mediated. Preliminary experiments suggest that ADP-stimulated PF4 release from platelets is impaired by CAP, DHC and OLDA whereas NADA enhances ADP-stimulated PF4 release.

Discussion. CAP, DHC, OLDA and NADA inhibit in vitro platelet aggregation, a mechanism that is not TRPV1 mediated nor due to a direct toxic effect on platelets. Vanilloids may inhibit platelet aggregation by interfering with granule release. Further studies are warranted.

Psychotropic drugs inhibit the acetylcholine receptor-operated potassium current (I_{K,ACH}) by different mechanisms in guinea-pig atrial myocytes.
Yukio Hara¹, Shinya Watanabe¹, Ryu Nakara¹, Takashi Matada¹, Yoko Asao¹, Muneyoshi Okada¹, Hideyuki Yamawaki¹. Lab of Vet Pharmacol, Kitasato Univ¹. Towada, Aomori 034-8628, Japan.

Introduction. Although psychotropics have a high therapeutic index and are generally safe agents, some of them produce cardiovascular complication by direct actions and indirect actions through nervous influences. Chlorpromazine, an antipsychotic drug, depressed cardiac repolarization and prolonged QTc resulting in increasing risk of malignant arrhythmia through inhibiting voltage-gated potassium channel. However, influences of psychotropics on ligand-gated potassium channel were not well examined.

Aim. Influences of six antipsychotics and three antidepressants on the acetylcholine receptor-operated potassium current (I_{K,ACH}) were examined. And mechanism of anticholinergic action was explored.

Methods. Whole-cell patch clamp method in freshly isolated guinea-pig atrial myocytes was used. I_{K,ACH} was induced by carbachol (CCh) or by an intracellular application of guanosine 5’-thio triphosphate (GTPγS). To elucidate the mechanism for inhibitory effect, the ratio of IC_{50} values for inhibition of GTPγS-activated I_{K,ACH} to CCh-induced I_{K,ACH} was calculated using following equation:

IC_{50} ratio = [IC_{50} for GTPγS-activated current] / [IC_{50} for CCh-induced current]

Results. Antipsychotics and antidepressants inhibited CCh-induced I_{K,ACH} in a concentration-dependent manner. The IC_{50} values (μmol/L) were as follows; chlorpromazine 0.53, clozapine 0.06, fluphenazine 2.69, haloperidol 2.66, sulpiride 42.3, thioridazine 0.07, amitriptyline 0.03, imipramine 0.22, and maprotiline 1.81. The drugs, except sulpiride, inhibited GTPγS-activated current with following IC_{50} values (μmol/L); chlorpromazine 1.71, clozapine 14.9, fluphenazine 3.55, haloperidol 2.73, thioridazine 1.90, amitriptyline 7.55, imipramine 7.09, and maprotiline 5.93. IC_{50} ratio for fluphenazine and haloperidol was close to unity. IC_{50} ratio for chlorpromazine, clozapine, thioridazine, amitriptyline, imipramine, and maprotiline was much higher than unity.

Discussion. Psychotropics studied suppressed I_{K,ACH}. Chlorpromazine, clozapine, thioridazine, amitriptyline, imipramine, and maprotiline are preferentially acting on muscarinic receptor. Fluphenazine and haloperidol may act on G protein and/or potassium channel.
Effect of diabetes on the production and vasoactivity of hydrogen sulfide in rat middle cerebral arteries.
Eloise Streeter, Emilio Badoer & Joanne Hart, Disc of Pharmaceutical Sciences, School of Medical Sciences, RMIT University², Bundoora, Victoria.

Introduction. Hydrogen sulfide is produced endogenously in vascular tissue by cystathionine-γ-lyase (CSE), and has both vasoregulatory and anti-oxidant effects. Little is known of H2S production or physiological role in cerebral vasculature, particularly in disease states such as diabetes, where there is increased oxidative stress.

Aims. To examine the effect of diabetes on H2S production and function in rat middle cerebral arteries.

Methods. Diabetes was induced with streptozotocin (50mg/kg, iv). Middle cerebral artery (MCA) function was examined using myography and superoxide anion (O2-) generation measured using NADPH(100μM)-dependent lucigenin-enhanced chemiluminescence. CSE mRNA expression was measured via RT-PCR and plasma sulfide and CSE activity were measured using a spectrophotometric assay.

Results. Diabetic rats had elevated blood glucose (P<0.05) and significantly reduced MCA endothelial function (Relaxation to the endothelium-dependent dilator bradykinin (100nM): Control: 48±7%; Diabetic: 30±4%; n=5, P<0.05). Vasorelaxation to exogenous H2S was unaffected in diabetic MCA and was elicited via a combination of K+, Cl- and Ca2+ channel modulation. Vasorelaxation to the H2S precursor L-cysteine was significantly enhanced in diabetic MCA (%Rmax: Control: 83±4; Diabetic: 97±3, n=7, P<0.01). Plasma sulfide, CSE activity and CSE mRNA were elevated in diabetes. MCA O2 production was increased in diabetes (O2 counts/mg (x10³): Control: 14.6±2.3; Diabetic: 43.0±9.4, n=9, P<0.05). This increase was attenuated by incubation with exogenous H2S (25.8±3.0, n=9, P<0.05).

Discussion. These data suggest that endogenous H2S production is upregulated in this model of diabetes. Vasorelaxation responses to exogenous H2S are preserved and exogenous H2S attenuates the enhanced MCA-generated O2- observed in the diabetic group. These data suggest that upregulation of endogenous H2S in diabetes may play a vasoprotective role.

Contractile recovery induced by pharmacological conditioning with GTN & cariporide at cardioplegia is associated with mitochondrial protective signaling in a model of donor heart preservation.
Jair Kwan¹, Ling Gao², Aoife Doyle², Peter Macdonald²,³, Mark Hicks²,⁴. ¹Free Radical Group, Heart Research Institute, Newtown, NSW; ²Cardiac Physiology and Transplantation Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW; ³Heart Lung Transplant Unit, ⁴Dept of Clin Pharmacol & Toxicol, St Vincent’s Hospital, Darlinghurst, NSW. (Introduced by Prof Ric Day, Dept of Clin Pharmacol & Toxicol, St Vincent’s Hospital, Darlinghurst, NSW)

Introduction: Activation of pro-survival pathways by pharmacological conditioning agents represents a novel approach to enhance post-storage function of donor hearts.

Aim: To audit the activation of the ERK, Akt and STAT3 pathways by the nitric oxide donor, glyceryl trinitrate, (GTN), and the sodium hydrogen exchange inhibitor, cariporide, after cold storage and warm reperfusion.

Methods: After baseline function was measured in isolated working rat hearts, they were arrested and stored for 6h in either Celsior, Celsior supplemented with 0.1mg/ml GTN, 10 μM cariporide or both agents combined. After reperfusion, cardiac function was re-measured then the tissue processed for immunoblotting or histology.

Results: GTN and cariporide alone or in combination significantly improved post-storage cardiac function (69 vs 20% of baseline cardiac output GTN+cariporide vs Celsior; P<0.05). Recovery was inhibited by stattic, an inhibitor of STAT3 phosphorylation. Significant increases in post-reperfusion necrosis and apoptosis in the Celsior group were abolished by inclusion of GTN, cariporide or both. Increased phosphorylation of ERK and its downstream target, Bcl2, after reperfusion was seen in groups stored in GTN cariporide or both along with increased phospho-STAT3 levels in the GTN/Cariporide group. No phospho-Akt increase was seen in any treatment.

Discussion: Phosphorylation of ERK and Bcl2 has been associated with activation of mitophagy (1), an important endogenous strategy for selection of highly functional mitochondria post reperfusion. The interaction of phospho-STAT3 with cyclophyllin D has also recently been implicated in decreasing the open probability of the mitochondrial permeability transition pore (2). Both processes are crucial for functional recovery of the heart after ischemia reperfusion injury. Importantly for potential application of this approach to clinical donor heart retrieval, the conditioning supplement(s) need only be added to the cardioplegic/storage solution.

**Pharmacological profiling of angiotensin II and bradykinin receptor heteromers.**

Elizabeth KM Johnstone¹, Mohammed Akli Ayoub¹,³ & Kevin DG Pfleger¹,². Lab for Mol Endocrinol – GPCRs, West Aust Inst for Med Res & Centre for Med Res, Univ of Western Australia¹, Nedlands, WA; Dimerix Bioscience², Nedlands, WA; Dept of Biochem, King Saud Univ³, Riyadh, Kingdom of Saudi Arabia.

Introduction: The renin-angiotensin system and the kallikrein-kinin system constitute two regulatory systems involved in the maintenance of blood pressure. Interactions between the two systems are numerous and have been extensively studied for decades. With the establishment of the concept of G protein-coupled receptor (GPCR) heteromerisation a new avenue of investigation has emerged. Several heteromers have already been identified including the heteromer between the type-1 and type-2 angiotensin II receptors (AT₁R and AT₂R) as well as the heteromer between the AT₂R and the bradykinin type-2 receptor (B₂R). Although the AT₁R-B₂R heteromer has also been described (AbdAlla et al, 2001), its existence remains contentious after a second study was unable to detect it in a variety of systems (Hansen et al, 2009).

Methods: This study has investigated interactions between the AT₁R, the AT₂R and the B₂R using various bioluminescence resonance energy transfer (BRET) techniques. The novel GPCR-heteromer identification technology (GPCR-HIT) enables detection of heteromers through ligand-dependent recruitment of GPCR interacting proteins such as arrestin. To investigate interactions between all three receptors, a modified GPCR-HIT assay which includes Venus complementation was employed. HEK293 cells were transfected with receptor or arrestin cDNA fused to either Rluc8 (variant of *Renilla* luciferase), Venus (variant of green fluorescent protein), or split Venus fragments 1 or 2.

Results: The results of GPCR-HIT studies between the AT₁R and the AT₂R suggest that an interaction may cause an inhibition of angiotensin II-induced arrestin recruitment to the AT₁R. The modified GPCR-HIT studies between all three receptors further support this, while also confirming the existence of the AT₁R-AT₂R heteromer and the AT₂R-B₂R heteromer.

Discussion: These studies highlight the complex interactions that occur between all three receptors, and suggest that these interactions likely lead to competition for formation of the heteromers.


Brain infarct volume after permanent focal ischemia is not dependent on Nox2 expression
Hyun Ah Kim\textsuperscript{1}, Vanessa H. Brait\textsuperscript{1}, Seyoung Lee\textsuperscript{1}, T. Michael De Silva\textsuperscript{1}, Henry Diep\textsuperscript{1}, Anja Eisenhardt\textsuperscript{1}, Grant R. Drummond\textsuperscript{1} & Christopher G. Sobey\textsuperscript{1}. Department of Pharmacology, Monash University\textsuperscript{1}, Clayton, VIC.

Introduction. Reactive oxygen species (ROS) generated by Nox2 oxidase are reported to contribute to infarct damage following cerebral ischemia-reperfusion. Experimental studies investigating mechanisms of post-stroke brain injury have mostly utilized models of ischemia with reperfusion. Hence, they are most relevant for elucidating the pathology occurring in ischemic stroke cases receiving clot-buster therapy or where there is spontaneous reperfusion. It is thus important to clarify if Nox2 oxidase is also a valid therapeutic target in ischemia without reperfusion.

Aims. This study aimed to pharmacologically characterise the role of T-type VOCC in different regions of the cerebral vasculature.

Methods. Vascular contraction was assessed via wire myography in ring segments of medulla interlobar arteries (internal diameter = 371±15 \( \mu \text{m} \)) and extrarenal arteries (antero- and posterior division, internal diameter = 629±21 \( \mu \text{m} \)) isolated from male Sprague Dawley rats. Vessels were exposed to graded, non-cumulative, sequential increases in depolarising potassium solution (10, 20, 40, 62 mM K\textsuperscript{+}) in the absence and presence of the selective T-type VOCC antagonist NNC 55-0396 (0.01-0.1 \( \mu \text{M} \), 30 min). To ascertain the role of T-type VOCC in agonist-induced contraction, endothelin-1 cumulative concentration-response curves were generated in the absence/presence of NNC 55-0396.

Results. NNC 55-0396 caused concentration-dependent inhibition of potassium-induced contraction; 0.1 \( \mu \text{M} \) decreased the maximum contraction in both interlobar (113±8 to 78±11%, \( n=4 \), \( P<0.05 \)) and extrarenal arteries (95±4 to 80±2%, \( n=5 \), \( P<0.05 \)). NNC 55-0396 (0.1 \( \mu \text{M} \)) also decreased the maximum contraction elicited by endothelin-1 (125±3, \( n=7 \) to 110±3%, \( n=3 \), \( P<0.05 \)) and increased the vasoconstrictor threshold (30-fold) in the interlobar but not extrarenal arteries. Basal tone was unaltered with the selective antagonist.

Discussion. T-type VOCC may contribute to vascular contraction in the renal vasculature. The extent of this contribution varies between regions. The contribution of T-type VOCC to the actions of other physiological vasoconstrictor and vasodilator agents remains to be characterised.


Region-dependent contribution of the T-type voltage-operated calcium channel to rat renal vascular contraction
Makhala M Khammy, James A Angus, Christine E Wright. Cardiovascular Therapeutics Unit, Dept of Pharmacol, Univ of Melbourne, Parkville, VIC.

Introduction. T-type voltage-operated calcium channels (VOCC) have been shown to be expressed in the systemic vasculature including the renal vasculature (Hansen et al., 2001). However, unlike the L-type VOCC, their contribution to smooth muscle tone and contraction is debated.

Aims. This study aimed to pharmacologically characterise the role of T-type VOCC in different regions of the renal vasculature.

Methods. Vascular contraction was assessed via wire myography in ring segments of medulla interlobar arteries (internal diameter = 371±15 \( \mu \text{m} \)) and extrarenal arteries (anterior and posterior division, internal diameter = 629±21 \( \mu \text{m} \)) isolated from male Sprague Dawley rats. Vessels were exposed to graded, non-cumulative, sequential increases in depolarising potassium solution (10, 20, 40, 62 mM K\textsuperscript{+}) in the absence and presence of the selective T-type VOCC antagonist NNC 55-0396 (0.01-0.1 \( \mu \text{M} \), 30 min). To ascertain the role of T-type VOCC in agonist-induced contraction, endothelin-1 cumulative concentration-response curves were generated in the absence/presence of NNC 55-0396.

Results. NNC 55-0396 caused concentration-dependent inhibition of potassium-induced contraction; 0.1 \( \mu \text{M} \) decreased the maximum contraction in both interlobar (113±8 to 78±11%, \( n=4 \), \( P<0.05 \)) and extrarenal arteries (95±4 to 80±2%, \( n=5 \), \( P<0.05 \)). NNC 55-0396 (0.1 \( \mu \text{M} \)) also decreased the maximum contraction elicited by endothelin-1 (125±3, \( n=7 \) to 110±3%, \( n=3 \), \( P<0.05 \)) and increased the vasoconstrictor threshold (30-fold) in the interlobar but not extrarenal arteries. Basal tone was unaltered with the selective antagonist.

Discussion. T-type VOCC may contribute to vascular contraction in the renal vasculature. The extent of this contribution varies between regions. The contribution of T-type VOCC to the actions of other physiological vasoconstrictor and vasodilator agents remains to be characterised.


POSTER ABSTRACTS
Neuroprotective effect of an angiotensin receptor-2 agonist following cerebral ischemia in vitro and in vivo
Seyoung Lee¹, Vanessa H Brait¹, Thiruma V. Arumugam², Megan A. Evans³, Hyun Ah Kim¹, Robert E. Widdop¹, Grant R. Drummond⁵, Christopher G. Sobey¹ and Emma S. Jones¹. Dept of Pharmacol, Monash University¹, Clayton, VIC. School of Biomedical Sciences, The University of Queensland², Brisbane, QLD.

Introduction. The renin-angiotensin system is known to be important in the development of a number of cardiovascular diseases. Intracerebral administration of the angiotensin II type 2 receptor (AT2R) agonist, CGP42112, is neuroprotective in a rat model of ischemic stroke.

Aims. To explore further its possible cellular target(s) and therapeutic utility, we firstly examined whether CGP42112 may exert direct protective effects on primary neurons following glucose deprivation in vitro. Secondly, we tested whether CGP42112 is effective when administered systemically in a mouse model of cerebral ischemia.

Methods. Primary cortical neurons were cultured from E17 C57Bl6 mouse embryos for 9 d, exposed to glucose deprivation for 24 h alone or with drug treatments, and percent cell survival assessed using trypan blue exclusion. Ischemic stroke was induced in adult male C57Bl6 mice by middle cerebral artery occlusion for 30 min, followed by reperfusion for 23.5 h. Neurological assessment was performed and then mice were euthanized and infarct and edema volume were analysed.

Results. During glucose deprivation, CGP42112 (1x10⁻⁸ M and 1x10⁻⁷ M) reduced cell death by ~30%, an effect that was prevented by the AT2R antagonist, PD123319 (1x10⁻⁶ M). Neuroprotection by CGP42112 was lost at a higher concentration (1x10⁻⁶ M) but was unmasked by co-application with the AT1R antagonist, candesartan (1x10⁻⁷ M). By contrast, Compound 21 (1x10⁻⁸ M to 1x10⁻⁶ M), a second AT2R agonist, had no effect on neuronal survival. Mice treated with CGP42112 (1 mg/kg i.p.) after cerebral ischemia had improved functional outcomes over vehicle-treated mice as well as reduced total and cortical infarct volumes.

Discussion. These results indicate that CGP42112 can directly protect neurons from ischemia-like injury in vitro via activation of AT2Rs, an effect opposed by AT1R activation at high concentrations. Furthermore, systemic administration of CGP42112 can reduce functional deficits and infarct volume following cerebral ischemia in vivo.

Endothelin and endothelin receptor antagonism in rat isolated cerebral arteries: relevance for subarachnoid haemorrhage
Yohannes A Mamo, James Ziogas, Paul F Soeding, Christine E Wright
Cardiovascular Therapeutics Unit, Dept of Pharmacol, Univ of Melbourne, Parkville, VIC.

Introduction. Delayed cerebral vasospasm following subarachnoid haemorrhage (SAH) is characterized by sustained narrowing of cerebral arteries. The pathological mechanism leading to vasospasm is not completely understood. Endothelin-1 (ET-1) is a key vasoconstrictor agent mediating development of vasospasm, where its concentration is increased in the cerebrospinal fluid (CSF) of SAH patients. Further, animal studies have revealed an increased sensitivity of cerebral arteries to ET-1 after SAH. There are two types of ET receptors - ETA and ETB. ETA receptors are expressed on vascular smooth muscle cells and mediate vasoconstriction, while ETB receptors, mainly located on vascular endothelium, mediate vasodilatation, except in pathological states when they may mediate vasoconstriction.

Aims. The aim of this study was to investigate regional variation in ET-1 responses in rat cerebral arteries.

Methods. Rat anterior cerebral (ACA), middle (MCA), posterior communicating (Pcom) and basilar (BA) arteries with respective internal diameters of 340±8, 320±9, 314±10 and 428±13 μm were dissected and ET-1 concentration-response curves were analyzed using wire myography.

Results. ET-1 caused potent contraction in all four cerebral arteries. The pEC⁰ of ET-1 in BA was 8.39±0.15, with no significant difference in ACA, MCA and Pcom. However, ACA, MCA and Pcom had a lower threshold than BA. Bosentan (a dual ETₐ and ET₇ receptor antagonist) shifted the ET-1 concentration-response curve to the right in all arteries except ACA at low concentration (0.1 μM); the rightward shifts were 1.4, 3.8, 4.0 and 3.2 fold in ACA, MCA, Pcom and BA, respectively. The selective ET₇ receptor agonist, sarafotoxin 6C, did not cause vasoconstriction, but induced concentration-dependent relaxation that was similar in all vessels.

Discussion. Rat cerebral arteries are highly sensitive to ET-1 vasoconstriction with some regional variation in the response to bosentan. Potent relaxant effects of sarafotoxin 6C indicate the presence of ET₇ receptors in intact cerebral vascular endothelium.
Chronic NaHS treatment protects vascular function by reducing oxidative stress in streptozotocin-induced diabetes in mice.

Hooi Hooi Ng & Joanne L Hart. School of Medical Sciences, RMIT Univ, Bundoora, Victoria, Australia.

Introduction. Hydrogen sulfide (H$_2$S) is endogenously produced in vascular tissue (Kimura 2011). H$_2$S is an antioxidant (Kimura 2011) and may be a useful therapeutic agent under conditions of increased oxidative stress. Aim. The aim was to investigate whether chronic treatment with the H$_2$S donor NaHS could elicit a vasoprotective effect in diabetes, where there is known to be increased oxidative stress (Shen 2010).

Methods. Diabetes was induced in male C57 mice with streptozotocin (60mg/kg daily, ip for 2 weeks) and confirmed by elevated blood glucose and HbA1C levels. Following a further 3 weeks, mice were then treated with NaHS (100μmol/kg/day) for 4 weeks, then tissues collected. Myography was employed to examine vascular reactivity and NO and H$_2$S bioavailability in thoracic aortae. Vascular superoxide levels were determined by the NADPH-dependent lucigenin-enhanced chemiluminescence assay. Plasma H$_2$S concentration and the activity of H$_2$S synthesising enzyme, cystathionine-γ-lyase (CSE) were measured by spectrophotometric assay.

Results. Vascular superoxide levels were significantly increased in diabetic aortae compared to control aortae (P<0.01). Daily NaHS treatment for 4 weeks in diabetic mice reduced superoxide production in diabetic aortae. ACh-mediated, endothelium-dependent vasorelaxation was significantly inhibited in diabetic aortae (P<0.05), but NaHS treatment restored the maximal relaxation to ACh. Vascular H$_2$S bioavailability and plasma H$_2$S concentration were reduced in diabetes, while liver CSE activity was significantly increased in diabetes (P<0.0001), however none of these parameters were affected by chronic NaHS treatment.

Discussion. These data suggest that NaHS acts as a scavenger of NADPH-induced superoxide and protects endothelial function in vivo in this model of oxidative stress.


Review of Epidemiology and Management of Atrial Fibrillation in Developing Countries

Tu Nguyen 1,2, Sarah Hilmer 1,2, Robert Cumming 2. Dept of Clin Pharmacol and Aged Care, Royal North Shore Hospital 1, Sydney, NSW; Sydney Medical School, Uni of Sydney 2, Sydney, NSW.

Introduction. Atrial fibrillation (AF) is the commonest sustained cardiac arrhythmia with a substantial impact on mortality and morbidity (Wolf P et al, 1999). Pharmacologic management of AF includes treatment of the underlying cause, anticoagulation to reduce the risk of stroke and systemic thromboembolism, and rate or rhythm control for symptomatic relief. Relatively little is known about AF in the developing world.

Aims. To examine in developing countries the prevalence of AF, the medical conditions associated with AF and the pharmacological management of AF.

Methods. A literature search was conducted via MEDLINE and EMBASE (1990-2012).

Results. A total of 70 articles were included in the review. The prevalence of AF in the general population ranged from 0.03% to 1.25% while the prevalence of AF in hospital-based studies varied according to the patient population studied (from 0.7% to 55.7%). Hypertension was the most common associated disease (10.3%-71.9%). The utilization of anticoagulants (coumarins) was highly variable (from 2.7% to 72.7%). No study was identified on the use of newer oral anticoagulants. Approximately half of AF patients using warfarin had therapeutic INR levels. There was a high prevalence of using rate control therapies (55.3% to 87.3%).

Discussion. The limited studies available suggest that in the developing world there is a significant prevalence of AF. Highly variable use of anticoagulants may be related to different health care and socioeconomic settings. More studies are needed to improve understanding of the epidemiology and management of AF in developing countries.

An allosteric enhancer of the adenosine $A_1$ receptor improves cardiac function following ischaemia in isolated murine hearts.

Roselyn B Rose$^{1}$, Paul J White$^2$, Peter J Scammells$^2$, Shane M Devine$^2$, Anna Butcher$^1$. School of Med Sci, Griffith Univ$^1$, Gold Coast, QLD; Monash Inst Pharmaceut Sci, Monash Univ$^2$, Parkville, VIC.

Introduction. Adenosine is released from tissues during the conditions of low oxygen tension. Stimulation of the adenosine $A_1$ receptor following an ischaemic episode has been shown to be cardioprotective (Butcher et al., 2007).

Aims. The effect of an allosteric enhancer of the adenosine $A_1$ receptors was investigated using an ischaemia-reperfusion protocol in murine isolated hearts.

Methods. Isolated hearts were perfused with Kreb-Henseleit solution gassed with 95%$O_2$; 5%$CO_2$ in Langendorff mode and electrically paced at 480 bpm. Following 20 mins equilibration and 20 mins global normothermic ischaemia, the allosteric enhancer VCP333 (1 $\mu$mol/L) or the partial adenosine $A_1$ receptor agonist VCP102 (10 $\mu$mol/L) were infused after 5 mins of reperfusion for 15 mins. Upon termination of the drug treatment, reperfusion continued for a further 40 mins.

Results. At the end of 60 mins reperfusion treatment with VCP333 or VCP102 the recovery of the left ventricular developed pressure was higher when compared to control group responses ($P<0.05$). Neither compound affected end diastolic pressure, coronary flow or $dP/dt_{max}$ values when compared to control tissues during reperfusion ($P>0.05$)

The infusion of VCP102 or VCP333 during reperfusion reduced cardiac troponin I efflux to 6.7% and 25% respectively of control heart efflux ($P<0.05$).

Conclusion. This data indicates that the allosteric enhancer of the adenosine $A_1$ receptor (VCP333) has similar characteristics to the adenosine receptor partial agonist VCP102 as it improves cardiac function and reduces myocardial cell death following an ischaemic episode.


The DPP-4 inhibitor linagliptan improves endothelium-dependent relaxation of rat mesenteric arteries in the presence of high glucose

Salheen M Salheen$^1$, Amanda Mather$^2$, Usha Panchapakesan$^2$, Carol Pollock$^2$ and Owen L Woodman$^1$. School of Medical Sciences, Health Innovations Research Institute RMIT University$^1$, Bundoora, VIC, Kolling Institute$^2$, St Leonards, NSW.

Introduction. The dipeptidyl peptidase-4 (DPP-4) inhibitors are a novel class of pharmacological agents used to treat hyperglycaemia that causes impairment of vascular endothelial function in type 2 diabetes. Both experimental and clinical studies indicate that DPP-4 inhibition may exert beneficial effects on the cardiovascular complications of diabetes independently of their glucose lowering effects.

Aim. To investigate the effect of linagliptin, a DDP-4 inhibitor, on the mechanism(s) of endothelium-dependent relaxation in rat mesenteric arteries in the absence and presence of high glucose (40 mM).

Methods. Endothelium-dependent and –independent relaxation to acetylcholine (ACh) (0.1 nM–10 $\mu$M) and sodium nitroprusside (SNP) (0.1 nM–10 $\mu$M) was determined in mesenteric arteries from Wistar rats pre-contracted with phenylephrine (10-100 nM) and exposed to normal (11 mM) or high (40 mM) glucose concentrations.

Results. Incubation of mesenteric rings with high glucose (40 mM) for 2 h caused a significant impairment of endothelium-dependent relaxation (ACh $pEC_{50}$ glucose: 11 mM = 7.76±0.20, 40 mM = 6.32±0.21, $p<0.05$), but did not affect SNP-induced relaxation. Co-incubation with linagliptin prevented the impairment of endothelium-dependent relaxation caused by high glucose (ACh $pEC_{50}$ 40 mM glucose + 1$\mu$M linagliptin = 7.20±0.07).

When the contribution of NO was abolished by N-nitro-L-arginine (L-NNA, 100 $\mu$M) plus a soluble guanylate cyclase inhibitor (ODQ, 10 $\mu$M), or the contribution of endothelium derived hyperpolarising factor (EDHF) was inhibited with TRAM-34 (1$\mu$M) plus apamin (1$\mu$M), the ACh-induced relaxation was significantly impaired by high glucose under both conditions suggesting that the contributions of both NO and EDHF were affected. Linagliptin significantly improved ACh-induced relaxation in the presence of both groups of inhibitors.

Discussion. Endothelium-dependent relaxation was impaired by high glucose but was significantly improved by linagliptin which preserved the actions of both NO and EDHF demonstrating that the vasoprotective actions are independent of any glucose lowering activity.
Direct stimulation of AT2R and the MasR prevents TNFα-induced endothelial inflammation.

Amanda K Sampson¹, Jennifer C Irvine¹, Tyrone A Barnes¹, Olivier Huet¹, Garry L Jennings¹, Robert E Widdop², Jaye PF Chin-Dusting¹. Vascular Pharmacology Department, Baker IDI Heart and Diabetes Institute¹, Melbourne, VIC; and Department of Pharmacology, Monash University², Clayton, VIC

Introduction. Activation of the angiotensin II type 1 receptor (AT1R) mediates pro-inflammatory effects with recent evidence demonstrating that activation of the angiotensin II type 2 receptor (AT2R) and Mas receptor (MasR) counteract some of the pro-inflammatory effects of AT1R stimulation such as cytokine release. Importantly, the potential anti-inflammatory properties of direct AT2R or MasR activation on the adhesion cascade remain unknown.

Aims. We aimed to examine whether direct activation of the AT2R and MasR elicits anti-inflammatory effects; specifically by reducing TNFα-induced leukocyte-endothelial cell adhesion and adhesion molecule expression.

Methods. Leukocyte-endothelial cell adhesion was examined in vitro (cultured HUVECs) and ex vivo (intact mouse thoracic aorta). Adhesion molecule (Intercellular Adhesion Molecule 1 (ICAM-1) and E-selectin) protein expression was assessed using flow cytometry.

Results. Direct stimulation of the AT2R (using Compound 21; C21 100 μM) and MasR (using AVE 0991; 100 μM) reduced TNFα-induced endothelial-leukocyte adhesion in vitro resulting in 54±5% (n=5, P<0.005) and 69±5% (n=4, P<0.01) of TNFα-induced adhesion (100%), respectively. These effects were abolished following concurrent treatment with the respective receptor antagonists (MasR agonist: A779, 10 μM, 110±5%, n=4 and AT2R agonist: PD 123319, 10 μM, 93±2%, n=4). Similarly, TNFα increased adhesion from 10±2 to 30±4 adhered leukocytes/field of view (FOV) in intact mouse aortae (n=12, P<0.001); which was completely prevented in aorta treated with C21 (10 μM, 11±4 adhered leukocytes/FOV, n=6, P<0.01). Furthermore, C21 reduced TNFα-induced ICAM-1 and E-selectin protein expression (n=3, P<0.005).

Discussion. This study provides the first evidence that direct activation of endothelial AT2R and MasR attenuates the inflammatory effects of TNFα in the setting of the vascular adhesion cascade.

Review of antithrombotic risk assessment tools for stroke prevention in atrial fibrillation patients

Yishen Wang¹, Beata V. Bajorek¹,²
Graduate School of Health-Pharmacy, The University of Technology Sydney¹, Sydney, NSW; Department of Pharmacy and Clinical Pharmacology, Royal North Shore Hospital², Sydney, NSW;

Introduction. Clinical guidelines advocate stroke prevention therapy in persons with Atrial fibrillation (AF), recommending antithrombotic agents (e.g., warfarin). However, the decision to initiate treatment is based on the risk (e.g., side-effects such as bleeding) versus benefit (prevention of stroke) of therapy, and this is often difficult to assess.

Aim. To identify and review available risk assessment tools to facilitate the optimal use of antithrombotic therapy for stroke prevention in AF.


Result. Overall, 18 tools were identified: 11 addressing stroke risk, 7 addressing bleeding risk. Among the stroke risk assessment tools (e.g., Framingham, SPAF, AFI, Birmingham), CHADS2 and CHA2DS2-VASc were the most commonly advocated tools, sharing common risk factors: age, hypertension, diabetes, previous stroke/transient ischaemic attack. Among the bleeding risk assessment tools (e.g., OBRI, ATRIA), HEMORR2HAGES and HAS-BLED were most commonly advocated, sharing common risk factors such as: age, previous bleeding, renal and liver impairment. Overall, all but 1 tool targeted the separate aspects of the risk versus benefit equation; only the Computerised Antithrombotic Risk Assessment Tool (CARAT) brings together individual risk assessment for both stroke and bleeding. None of the other tools consider other key factors in decision-making regarding antithrombotic therapy, particularly those increasing the risk of medication misadventure with treatment (e.g., function, cognition, drug interactions, medication adherence, medication management capabilities).

Discussion. Although, separate tools are available to assess stroke risk and bleeding risk independently, but they do not estimate the relative risk versus benefit of available treatment options in an individual patient. Also, these separate tools seldom consider key medication safety aspects of prescribing treatment. More effort is needed to synthesise these separate risk assessments, and integrate key medication safety issues, particularly in view of the introduction of new anticoagulants into practice.
Allosteric Modulation of a Chemogenetically Modified G Protein-Coupled Receptor
Alaa Abdul-Ridha, J. Robert Lane, Patrick M. Sexton, Meritxell Canals and Arthur Christopoulos. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences and Department of Pharmacology, Monash University, Parkville, Victoria, 3052, Australia.

Introduction. DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) are chemogenetically modified muscarinic acetylcholine receptors (mAChRs) that have minimal responsiveness to ACh, but are potently and efficaciously activated by an otherwise inert synthetic ligand, clozapine-N-oxide (CNO). DREADDs have been used as tools for selectively modulating signal transduction pathways in vitro and in vivo. Recent comprehensive studies have validated how the pharmacology of a CNO-bound DREADD mirrors that of an ACh-bound wild-type (WT) mAChR. However, nothing is known about whether this equivalence extends to the allosteric modulation of DREADDs by small molecules.

Aims. To investigate the actions at an M₁ DREADD of BQCA, a positive allosteric modulator of ACh binding and function that is known to behave according to a simple two-state mechanism at the WT receptor.

Methods. Radioligand binding studies and a range of intracellular functional assays were performed on Chinese Hamster Ovary (CHO) cell expressing either WT or DREADD mAChRs.

Results. 1. Allosteric modulation of the CNO-bound DREADD receptor is not equivalent to the corresponding modulation of the ACh-bound WT receptor. 2. BQCA engenders stimulus bias at the M₁ DREADD, having differential types of cooperativity depending on the signaling pathway. 3. The modulation of ACh itself by BQCA at the DREADD is not compatible with the two state model that has been previously applied to the M₁ WT.

Discussion. The results indicate that caution must be exercised when interpreting studies of allosteric modulation using DREADDs.

The effect of morphine on the growth and dissemination of breast tumour in mice
Banafsheh Afshar-Imani¹, JoAnne Baran², Peter J Cabot¹, Marie-Odile Parat¹,². School of Pharmacy, Univ of Queensland¹, Woolloongabba, Australia; Dept of Anesthesia Res, Cleveland Clinic², Cleveland, OH.

Introduction. Appropriate pain management during and after cancer surgery may play a role in prevention of tumour recurrence and metastasis. Opioids are proven to be highly effective perioperative analgesics and are widely used in cancer surgery patients.

Aims. Using a mouse syngeneic model of breast cancer, we studied the effect of morphine on tumour growth and dissemination to lungs. We investigated in an in vitro co-culture model the effect of morphine on the interaction of breast tumour cells with non-malignant cells present in the tumour microenvironment, namely macrophages and endothelial cells.

Methods. Morphine was injected intraperitoneally (10mg/kg) to mice (n=8) every 12h for 3 days and its effect on murine 4T1 breast tumour cell dissemination to the lungs was measured 18 days after tumour inoculation. In the in vitro studies, 4T1 breast cancer cells, RAW264.7 macrophages, H5V endothelial cells and co-cultures of 4T1 with either macrophages or endothelial cells were treated with morphine (0.1-10 μM). The level of extracellular matrix (ECM) degrading enzymes, matrix metalloproteinase-9 (MMP-9) and urokinase-like plasminogen activator (uPA) as well as tissue inhibitors of matrix metalloproteinases (TIMPs) were measured in the conditioned media using in-gel zymography.

Results. Morphine treatment caused a reduction in breast tumour growth and tumour cell dissemination to the lungs. Morphine treatment also caused a reduction in circulating MMP-9 and uPA. In co-cultures of 4T1 cells with endothelial cells or macrophages, the level of matrix proteases was increased, and so was the level of TIMPs. Morphine treatment reduced the level of MMP-9 and increased its endogenous inhibitor, TIMP-1 in co-cultures but not cells grown individually.

Discussion. Our data suggest that morphine treatment could decrease tumour growth and dissemination in mice and that this anti-tumour effects are mediated at least in part through modulation of paracrine communication between cancer cells and tumour infiltrating cells.
Altered purinergic receptor calcium signalling associated with hypoxia in MDA-MB-468 breast cancer cells
Iman Azimi1, Hannah Beilby1, Felicity M Davis1, Sarah J Roberts-Thomson1, Gregory R Monteith1. School of Pharmacy, The Univ of Queensland1, Brisbane, QLD.

Introduction. Hypoxia is a common feature of the microenvironment of some breast cancers. Hypoxia can induce epithelial-mesenchymal transition (EMT) a process that can convert breast cancer cells into a more invasive phenotype. We have previously shown an association between epidermal growth factor (EGF)-mediated EMT and alterations in purinergic receptor-mediated calcium signalling and levels of purinergic receptor mRNA (e.g. up-regulation of P2X5).

Aims. 1. To compare changes in ATP-induced calcium transients in MDA-MB-468 breast cancer cells in normoxia and hypoxia. 2. To assess mRNA levels of a panel of purinergic receptors in MDA-MB-468 cells in normoxia and hypoxia.

Method. MDA-MB-468 cells were placed in a hypoxic incubator (0.1% O2) for 24 hours at 37°C. Cytosolic free Ca2+ ([Ca2+]CYT) levels were assessed using a Fluorescent Imaging Plate Reader (FLIPR). Changes in mRNA levels were evaluated by quantitative real-time RT-PCR.

Results. Hypoxia-induced EMT was confirmed by changes in EMT markers including vimentin, N-cadherin, snail, and CD24. The nature of the cytosolic calcium response to ATP was altered with hypoxia (e.g. EC50 0.5 μM in normoxia vs 1.3 μM in hypoxia). Significant changes in mRNA levels of some purinergic receptors in response to hypoxia were observed, however, these were often different from those previously observed with EGF-induced EMT.

Discussion. Hypoxia-induced EMT alters ATP-mediated calcium signalling and mRNA levels of purinergic receptors in MDA-MB-468 breast cancer cells. Further studies are required to assess the significance of altered purinergic receptor-mediated Ca2+ signalling associated with the acquisition of EMT.

Functional selectivity at the adenosine A1 receptor: Implications for cytoprotection.
Jo-Anne Baltos, Arthur Christopoulos & Lauren T May. MIPS and Dept of Pharmacol, Monash University, Parkville, VIC.

Introduction. Adenosine A1 receptor (A1AR) stimulation is protective in a number of cardiovascular and neuronal conditions, however, current therapeutic targeting is limited due to bradycardia (Jacobson & Gao, 2006). Remarkably, the novel A1AR agonists, VCP28 and VCP746, retain cytoprotective signaling in the absence of bradycardia, a phenomenon suggestive of functional selectivity (Kenakin et al., 2012; Urmaliya et al., 2010).

Aims. To delineate A1AR-mediated cytoprotective signal transduction and quantify the ability of the A1AR ligands, NECA, R-PIA, VCP28 and VCP746 to promote cytoprotective signal transduction.

Methods. A propidium iodide-based assay assessed A1AR agonist-mediated cytoprotection of CHO cells stably expressing the human A1AR (CHO-A1) after 24-hour serum starvation in the absence or presence of pharmacological inhibitors. Phosphorylation of ERK1/2 and AKT, calcium mobilization and cAMP accumulation were determined using fluorescence and luminescence approaches. Functional selectivity was quantified as described previously (Kenakin et al., 2012).

Results. A1AR agonist stimulation of CHO-A1 cell survival (n=9) was dependent on Gi/o protein stimulation, phosphorylation of ERK1/2 and AKT1/2/3 and PKC activation (n=3). Functional assays demonstrated that each A1AR agonist mediated a robust increase in ERK1/2 and AKT1/2/3 phosphorylation, calcium mobilization and inhibition of cAMP accumulation (n=3-6). In contrast to R-PIA and NECA, VCP28 and VCP746 showed functional selectivity with respect to calcium mobilization (p<0.05, one-way ANOVA, Tukey Post Hoc test).

Discussion. A1AR agonists stimulate CHO-A1 cell protection via Gi/o protein, ERK1/2, AKT1/2/3 and PKC dependent mechanisms. Functionally selective signalling may underlie the preferential physiological profile observed for VCP28 and VCP746.

**Can interference of GIP activity modulate GPCRs for therapeutic gains?**

Kenneth A Chinkwo 1, Ian M Coupar 2 and Helen R Irving 2

School of Biomedical Sciences, Charles Sturt University 1, Wagga Wagga NSW; Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University 2, Parkville VIC.

Introduction: GPCRs are a large family of cell surface proteins participating in signal transduction where the C-terminus recruits GPCR Interacting Proteins (GIPs) that regulate GPCR function. The 5-HT 4 (a, d, e, f and g) receptor splice variants possess canonical type 1 or type 2 PDZ domains predicted to interact with various GIPs which could influence their modulatory and prokinetic functions in the intestine (Coupar et al. 2007).

Aims: To investigate the distribution of 5-HT 4 receptors and GIPs in the guinea pig intestine and secondly to determine if human 5-HT 4 receptors and specific GIPs interact using an in vitro cell system.

Methods: RT-PCR, western and immunoflourescence analysis were used to investigate transcript and protein expression. N-terminal FLAG tagged 5-HT 4 receptor splice variants and Lin 7 homologues with C-terminal tagged V5, c-Myc and HA constructs were generated and expressed in COS-7 cells to study protein interactions.

Results: 5-HT 4 receptors, GRKs and Lin 7 homologues were expressed in the guinea pig intestine. Lin 7 homologues and 5-HT 4 receptors co-localized in cell lines and the 5-HT 4a receptor and Lin 7 were co-immunoprecipitated.

Discussion: We have previously shown that 5-HT 4 receptors and the GIPs, GRKs and Lin7 homologues, are found in the human colon (Chetty et al. 2009). Our data here indicates that this also occurs in the guinea pig and we demonstrate that it may be possible for 5-HT 4 receptor and Lin 7 to interact in the same cells. The results suggest that Lin 7 could be a potential target to modulate 5-HT 4 receptor function. This would be of particular relevance when 5-HT 4 splice variant expression is altered as occurs in cancerous tissue (Cartier et al. 2005).

Coupar IM et al. 2007 Curr Neuropharmacol 5: 224-31

**Activation and acute desensitization of μ-opioid receptor wild-type and mutants with deleted phosphorylation sites**

Marina Santiago 1, YanPing Du 2, Macdonald Christie 2, Mark Connor 1
Australian School of Advanced Medicine, Macquarie University 1, Sydney, NSW; Pharmacology, University of Sydney 2, Sydney, NSW

Introduction: Opioids are widely used clinically because of their unique analgesic properties, largely mediated by activation of the μ-opioid receptor (MOR). Understanding the molecular mechanisms underlying MOR regulation is important for developing pain relieving drugs that will decrease unwanted effects, dependence and tolerance.

Aims: To investigate the mechanisms of acute MOR desensitization with an emphasis on receptor phosphorylation.

Methods: Wild-type mouse MOR (MOR-WT) and MOR with mutations of c-terminus phosphorylation sites were stably transfected in AtT-20 cells and grown in 96 well plates. MOR signalling was measured using a proprietary membrane potential dye (Molecular Devices) in a Flexstation 3. MOR desensitization was quantified using a high “challenge” concentration of agonist added after the start of the desensitizing stimulus. Heterologous desensitization was assessed with somatostatin.

Results: Activation of MOR hyperpolarized AtT-20 cells, and the hyperpolarization waned over time. Morphine and [d-Ala2,N-MePhe4,Gly-ol]-enkephalin (DAMGO) activated each MOR variant with a similar potency and signaling at all variants was inhibited by prolonged exposure to morphine. In cells expressing MOR-WT, morphine (1μM) produced a 71±2% inhibition of the hyperpolarization caused by a subsequent application of 10μM morphine (t1/2 343s, 95% CI 264-490s). Morphine (1μM) inhibited the response to somatostatin (1μM) by 29±1%, with a similar timecourse. Met-enkephalin (1μM) produced a maximum 58±2% inhibition of a subsequent application of ME (10μM), and a 47±3% decrease in the response to somatostatin (n=5). Pretreatment of MOR-WT cells with the protein kinase C activator phorbol 12-myristate 13-acetate (1μM) or the protein kinase inhibitor staurosporine (1μM) did not significantly affect morphine potency or desensitization (n=5).

Discussion: These results support the idea that MOR desensitization is rapid and not necessarily dependent on protein kinase C. In addition, desensitization persists after deletion of several key phosphorylation sites in the c-terminal tail of the receptor.
Selective GPCR signalling opens TRPV4 expressed in HEK293 cells
William G Darby1, Fe C Abogadie2, Daniel P Poole2, Nicholas Veldhuis2, Michael Lew1, Nigel Bunnett2, Peter McIntyre4. Pharmacol Dept, Melbourne University1, Parkville, VIC; Monash Institute of Pharmaceutical Sciences3, Parkville, VIC; Research Institute, RMIT University4, Bundoora, VIC.

Introduction. Activation of muscarinic receptors in mouse vascular endothelial cells leads to the opening of the ion channel TRPV4 (Adapala et al, 2011). We hypothesized that other GPCRs might also be able to open TRPV4.

Aims. To investigate the ability of endogenous or exogenous GPCRs expressed in HEK293 cells to open TRPV4.

Methods. We generated HEK293 cell lines expressing wild type human TRPV4 (TRPV4) and compared the rise in intracellular calcium ([Ca2+]i) evoked by agonists for different GPCRs expressed in HEK293 cells (e.g. PAR1 and PAR2 and P2Y receptors) with non transfected cells (Nt), using a FURA-2 calcium influx assay in a Fluorimeter. Angiotensin II (ANGII) receptor 1 (AT1R) is not expressed in HEK293 cells, so it was transiently transfected into Nt and TRPV4 and responses to angiotensin, measured using fluorescence microscopy.

Results. PAR1 and PAR2 receptors were activated with SFLLR-NH2 and SLIGRL-NH2, respectively and evoked a transient increase of [Ca2+]i in Nt that returned to baseline 35s after stimulation, whereas, in TRPV4 cells, there was a sustained increase in [Ca2+]i measured 60s after stimulation (p<0.01, n=4, t test). The AT1R receptor showed a similar difference between Nt and TRPV4 with the sustained response in TRPV4. We did not observe opening in response to P2Y activation by ATP.

Discussion. We show that GPCR opening of TRPV4 in HEK293 occurs with activation of PAR1, PAR2 and AT1R but not P2Y receptors, showing that a transient increase in [Ca2+]i alone, is not sufficient to open TRPV4.


The binding mode of SB269652: a novel bitopic ligand at the dopamine D2 receptor
Christopher J Draper-Joyce1, Jeremy Shonberg2, Laura M Lopez1, Ben Capuano2, Arthur Christopoulos1, Robert Lane1, Drug Discovery Biology1 and Medicinal Chemistry2, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC

Introduction. To date all clinically effective antipsychotics target the D2 dopamine receptor (D2R) by competing with the neurotransmitter dopamine for the ‘orthosteric’ binding site. SB269652 was recently identified as the first negative allosteric modulator of the D2R (Silvano et al. 2010). We have progressively truncated fragments of this ligand, demonstrating that SB269652 has a ‘bitopic’ (simultaneous allosteric/orthosteric) mode of interaction with the D2R.

Aim. To gain further insight as to the specific receptor residues important for binding and function of this ligand

Methods. We selected and mutated residues in both orthosteric and putative allosteric sites of the D2R and screened these mutants in both functional (D2R mediated ERK1/2 phosphorylation) and radioligand binding assays ([3H]-spiperone)

Results. Mutation to alanine of two residues predicted to interact with the allosteric moiety of SB269652, Glu95 and Val91, caused a significant decrease in the negative cooperativity exerted by SB269652 upon dopamine (log\(k_{\text{D}}\)Glu95Ala = -0.32±0.14; log\(k_{\text{D}}\)Val91Ala = -0.48±0.16; n = 3, P<0.05) as compared to the wild type receptor (log\(k_{\text{D}}\)WT = -1.20±0.12). Similarly, mutation of Ser194 within the orthosteric site to alanine caused a significant decrease in negative cooperativity (log\(k_{\text{D}}\)Ser194Ala = -0.52±0.09, n = 3, P<0.05).

Discussion: These data demonstrate that SB269652 makes key interactions both within the orthosteric site and in an allosteric site at the top of transmembrane domain 2. As such this study provides validation of a bitopic mechanism of action for SB269652 and reveals the location of a novel allosteric site within the D2R.

Antitumour actions of the synthetic \( \omega-3 \) 17,18-epoxyeicosanoic acid in breast cancer cells
Herryawan RE Dyari, Pei H Cui, Tristan Rawling and Michael Murray. Pharmacogenomics and Drug Development, Faculty of Pharmacy, University of Sydney, NSW 2006, Australia.

Introduction. Unlike \(-6\) polyunsaturated fatty acids (PUFA), which promote the growth and spread of tumours, \( \omega-3 \) fatty acids decrease tumourigenesis. We have found previously that the CYP-derived \( \omega-3 \) epoxide of the naturally occurring eicosapentaenoic acid inhibits cell proliferation by down-regulating cyclin D1 (Cui et al., 2011). In contrast, isomeric epoxides formed by the action of CYPs on non-\( \omega-3 \) olefinic bonds in \( \omega-3 \) and \( \omega-6 \) PUFA stimulated cell growth.

Aims. This study evaluated the anti-tumour actions of \( \omega-3 \) 17,18-epoxyeicosanoic acid (\( \omega-3 \) EEA), a saturated monoepoxide that was synthesized to circumvent the formation of protumourigenic PUFA epoxides, in breast cancer cells.

Methods. \( \omega-3 \) EEA was synthesized from 17,18-eicosenoic acid (Cui et al., 2012) by treatment with m-chloroperbenzoic acid. MDA-MB 231 and MDA-MB 468 cells were treated with several concentrations of \( \omega-3 \) EEA for varying times. Endpoints relating to proliferation (MTT reduction, cell cycle kinetics and cyclin D1/E expression) and apoptosis (caspase-3 activity, caspase-3/9 and PARP cleavage, sub-G1 analysis, annexin V staining, and expression of cytochrome c and several Bcl family proteins) were estimated.

Results. \( \omega-3 \) EEA decreased proliferation and activated apoptosis in MDA-MB 231 and, to a lesser extent, the less aggressive MDA-MB 468 cells in a concentration- and time-dependent fashion. The cell cycle was arrested at G0/G1 phase due to a decrease in cyclin D1 expression. The Bak/Bcl-2 ratio was increased in treated cells.

Discussion. These findings suggest that the mechanism of breast cancer cell growth inhibition by \( \omega-3 \) EEA involves activation of the intrinsic (mitochondrial) death pathway and cell cycle arrest. \( \omega-3 \) EEA may be the prototype of a novel class of anticancer agents based on \( \omega-3 \) PUFA epoxides.

Cui et al. (2011) Br J Pharmacol 162, 1143-1155
Cui et al. (2012) J Med Chem 55, 7163-7172

Identification of novel allosteric modulators of the \( \alpha_{1A} \) and \( \beta_{2} \) adrenoceptors
Angela M Finch1, Junli Chen1, Erica Leonar1, Tony Ngo1, Renate Griffith1. 1Dept of Pharmacology, School of Medical Sciences, UNSW, Sydney, NSW.

Introduction. Current adrenoceptor (AR) allosteric modulators are either peptidic or have significant off-target affinity, such as the \( \beta_{2} \)AR positive allosteric modulator (PAM), the “MA” fragment of the bitopic ligand THRX-198321, which is also a muscarinic receptor antagonist (Steinfeld et al, 2011).

Aim. To identify novel small molecule allosteric modulators of the \( \alpha_{1A} \) and \( \beta_{2} \)AR.

Methods. Allosteric modulators were docked into a \( \beta_{2} \)AR crystal structure (3P0G) (MA, THRX-198321) or an \( \alpha_{1A} \)AR homology model (C9). A receptor-ligand pharmacophore was generated based the MA/\( \beta_{2} \)AR docking data and a structure-based pharmacophore were developed in Discovery Studio and used to screen the SPECS database. COS-1 membranes expressing \( \alpha_{1} \) or \( \beta_{2} \)AR where used in radioligand dissociation assays.

Results. Docking studies predicted H296, K305 and Y308 to form interactions with MA. H296A, K305A and Y308A mutations resulted in an increased dissociation rate of [\( ^{3} \)H]dihydroalprenolol (DHA) (\( k_{diss} \)) compared to wildtype. Y308A also altered MA’s (1mM) ability to decrease [\( ^{3} \)H]DHA dissociation (wildtype \( k_{diss}/k_{diss} \) 56±2.5%; Y308A \( k_{diss}/k_{diss} \) 75±2.7%, n=3 p<0.05). Seven compounds were chosen from the SPECS database. Preliminary testing has shown that compound 4 displays properties of a \( \beta_{2} \)AR PAM, decreasing the \( k_{diss} \) of [\( ^{3} \)H]DHA ([\( ^{3} \)H]DHA, 0.074 min \(^{-1} \), + Compound 4 (100\( \mu \)M), 0.019 min \(^{-1} \)). We have also identified novel \( \alpha_{1A} \)AR negative allosteric modulators; a symmetrical bis-4-aminoquinoline (C9) and 4-aminoquinoline (C9 30\( \mu \)M, \( k_{diss}/k_{diss} \) 529±29%; 4-aminoquinoline 300\( \mu \)M, \( k_{diss}/k_{diss} \) 206±15%, n=3). The docking studies suggest that C9 interacts with both the orthosteric binding site and residues at the top of transmembrane helix II.

Discussion. We have identified amino acids associated with allosteric effects in the extracellular regions of \( \alpha_{1A} \) and \( \beta_{2} \)AR. Novel allosteric compounds have been identified which can be further developed into pharmaceutical agents targeting the \( \alpha_{1A} \) and \( \beta_{2} \) AR.

References
Inhibition of human haematological malignant cell line growth by capsaicin is not TRPV1-mediated
Sofia Omari, Dale A Kunde, Murray Adams, Dominic P Geraghty. School of Human Life Sciences, Univ of Tasmania, Launceston, TAS.

Aim. Transient receptor potential vanilloid-1 (TRPV1) is a non-selective cation channel activated by a variety of endogenous and exogenous stimuli, including the major active component of ‘hot chilli peppers’, capsaicin. Recent evidence suggests that capsaicin induces apoptosis and inhibits cell proliferation, although this has not been extensively investigated in haematological malignancies. The aims of this study were to: 1) investigate the whether capsaicin kills human haematological malignant cells, and if so, 2) whether this action was TRPV1-mediated.

Methods. THP-1 (acute monocytic leukaemia), U266B1 (myeloma) and U937 (histiocytic lymphoma) cells were exposed to increasing concentrations of capsaicin (8-1000 μM) in the presence and absence of TRPV1, and cannabinoid 1 and 2 receptor (CB1, CB2; 0.1-100 μM) antagonists. Cell metabolic activity (indicative of viability) was measured after 24hrs using the alamarBlue® method (resazurin reduction assay).

Results. Capsaicin reduced viable THP-1, U266B1 and U937 cell numbers in a concentration-dependant manner. A biphasic effect was observed on THP-1 cells [EC50 and IC50 (95% CI) = 32.9 (19.9-54.3) and 219 (144-246) μM]. SB452533 and AM251 (100 μM) suppressed the capsaicin-induced increase in THP-1 cell activity (P<0.001). U266B1 cells were more resistant to capsaicin than THP-1 and U937 cells. Cell activity was significantly inhibited by capsaicin in U937 compared to U266 cells (IC50: 197 vs. 431 μM, respectively, P<0.008). AM251 and SB452533 appeared to act as partial agonists and displayed a synergistic effect with capsaicin in U937 cells.

Discussion. THP-1, U266B1 and U937 cells responded differently to capsaicin. TRPV1, CB1 and CB2 antagonists did not affect capsaicin-induced changes in U266B1 cell activity although CB1 and CB2 receptors appeared to mediate an increase in cell activity in THP-1. We conclude that capsaicin inhibits the viability of haematological malignant cells through a non-TRPV1-dependent mechanism.

Investigation of activation mechanism of TRPA1 ion channel
Liuqiong Gu1, William J Redmond2, Michael Lew1,Mark Connor2, Peter McIntyre3 Dept. of Pharmacol., University of Melbourne1, Parkville, VIC; Australia school of Advanced Medicines, Macquarie University2, NSW; Health Innovations Research Institute, RMIT University3, Bundoora, VIC.

Introduction. The Transient receptor potential ion channel TRPA1 is expressed in a subset of nociceptive neurons. It can be activated by noxious cold and by pungent electrophilic compounds like allyl isothiocyanate (AITC), the pungent component of wasabi and mustard oil and non-reactive compounds such as menthol (Story GM et al. 2003). Recent studies demonstrated that electrophilic compounds activate TRPA1 by covalent binding to the N-terminal cysteine (C) and lysine (K) residues (Hinman et al., 2006; Macpherson et al., 2007).

Aims. The aim of this study is to further investigate the role of the implicated N-terminal C and K residues in the activation of TRPA1 by both electrophilic and non-reactive compounds.

Methods. Cell lines expressing human TRPA1 or mutants of the N-terminal C and K residues were generated in TRex HEK293 cells (Invitrogen). Intracellular calcium imaging assays using FURA2 in a plate-reading fluorimeter (FlexStation 3, Molecular Devices) were used to characterize responses to compounds.

Results. Our data suggest that the N-terminal C and K residues are important for activation of TRPA1 by both electrophilic and non-electrophilic compounds. K708 is crucial for menthol activation.

Story GM et al. (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112(6):819-829.
A methanolic extract of propolis collected from the Australian native stingless bee, *Tetragonula carbonaria*, scavenges free radicals and inhibits 5-lipoxygenase activity *in vitro*
Karina D Hamilton¹, Peter R Brooks¹, Helen M Wallace¹, Steven Ogbourne¹ & Fraser D Russell¹. Faculty of Science, Health, Education & Engineering, Univ Sunshine Coast¹, Maroochydore, QLD.

Introduction. Propolis is a resinous material produced by bees from plant exudates, beeswax and salivary secretions. Studies conducted on global sources of propolis have indicated its anti-inflammatory and anti-oxidant effects (Araujo et al, 2012; Kumazawa et al, 2004); however, similar properties within propolis collected from Australian native stingless bees (*Tetragonula carbonaria*) have not been extensively studied.

Aims. To examine the potential of a *T. carbonaria* propolis extract to scavenge free radical species and inhibit 5-lipoxygenase activity *in vitro*.

Methods. Propolis collected from 40 *T. carbonaria* hives in South-East Queensland was homogenized and extracted in 2:1 methanol:hexane. The methanolic extract was dried and reconstituted for use in bioassays. Anti-oxidant activity of the extract (1-500 µg/mL in methanol) was assayed colorimetrically using 1,1-diphenyl-2-picrylhydrazyl (DPPH; 100 µmol/L), and by HPLC analysis of the extract (2 mg/mL in acetonitrile) spiked with 2,2'-azobis-2-methyl-propanimidamide (AAPH; 80 mg/mL; 40°C; 8 h). The effect of the extract (1-500 µg/mL in dimethyl sulfoxide) on the oxidation of linoleic acid by 5-lipoxygenase was examined using a cell-free colorimetric assay and by examination of Michaelis-Menten kinetics.

Results. A methanolic extract of *T. carbonaria* propolis dose-dependently scavenged DPPH (EC₅₀=27.0±2.34 µg/mL; n=3). HPLC analysis of the AAPH-spiked extract revealed several polar compounds potentially responsible for this bioactivity. The extract also inhibited 5-lipoxygenase activity in a dose-dependent manner (IC₅₀=70.0±19.84 µg/mL; n=3). Linoleic acid oxidation by 5-lipoxygenase (Kₐ₅₀=115.0±7.34 µmol/L; Vₘₐₓ=0.08±0.006 absorbance units/min) was significantly altered in the presence of 100 µg/mL extract (Kₐ₅₀=71.3±10.35 µmol/L; Vₘₐₓ=0.04±0.002 absorbance units/min; n=3; P<0.05), suggesting mixed enzyme inhibition. Solvent controls had no activity in any assay.

Discussion. A polar extract of Australian native stingless bee propolis displayed free radical-scavenging activity and mixed inhibition of 5-lipoxygenase activity *in vitro*. Further bioactivity-guided fractionation and chemical analysis of the extract are required to isolate and identify the constituent compounds responsible for these properties.


Opioid inhibition of cAMP in an optimised cell based assay
Dilanthi R Herath, Michael Morgan, Amitha K Hewavitharana, P Nicholas Shaw, Peter J Cabot.
School of Pharmacy, The University of Queensland, Brisbane, QLD 4072.

Introduction. Opioid peptides are capable of providing efficacious analgesia through their interaction with opioid receptors; activation of opioid receptors changes cell excitability through a number of downstream mechanisms. A major mechanistic pathway involves the inhibition of adenylyl cyclase which leads to decreased levels of cyclic adenosine monophosphate (cAMP). Therefore, evaluation of cAMP inhibition potencies of peptides can provide an insight to their analgesic potencies.

Aims. This study was aimed at optimising the experimental conditions for the determination of cAMP inhibition in a cell based system.

Methods. Beta endorphin (β-END) was used as a prototypical opioid peptide agonist and the study utilised a cAMP Alphascreen method (PerkinElmer) and cultured HEK cells overexpressing mu opioid receptors. The cell concentration and extent of activation by forskolin were examined and then the cAMP inhibition potencies of β-END and fentanyl were assessed. Finally, opioid specificity was confirmed by blockade with the non-selective opioid antagonist naloxone.

Results. 20,000 cells/well, 30 µM forskolin and 30 min incubation time were found to be the optimal conditions for cAMP assay. Fentanyl and β-END both inhibited forskolin increased cAMP with IC₅₀ values of 0.01µM and 0.2µM respectively. Prior incubation with 100 µM of naloxone prevented the cAMP inhibition of both β-END and fentanyl.

Discussion: This study describes an efficient and accurate method for the comparison of both exogenous and endogenous opioid efficacy in a cell-based system. Fentanyl is a clinically utilised mu opioid agonist whilst β-END is one of the most ubiquitous endogenous peptides. The optimised assay conditions determined in this study allowed for the comparison of β-END potency with that of fentanyl and for the demonstration of opioid receptor specificity.
Activity of a novel alpha-conotoxin LsIA, isolated from Conus limpurisi
Mr Marco Inserra¹, Dr Irina Vetter¹, Dr Andreas Brust¹, Mr Shiva Nag², Dr Anton Grishin², Prof David Adams², Prof Paul Alewood¹, Prof Richard Lewis², Institute for Molecular Biosciences¹, Brisbane, QLD; Health Innovation Research Institute², Melbourne, VIC

LsIA is an α-conotoxin that has been isolated and purified from the crude venom of the vermivorous conesnail, Conus limpurisi, found off the coast of south east Queensland, Australia. LsIA contains a 4/7 cysteine motif which is common among α-conotoxins and the Ser-Xaa-Pro-Xaa motif associated with this family of conopeptides. Taking into account the linage of α-conotoxins it would be expected that LsIA antagonises neuronal nicotinic acetylcholine receptors (nAChRs). This α-conotoxin inhibits rat α₃β₂ and α₁ nAChRs. Interestingly, native LsIA does not appear to block human α₃β₂ while the analogue [R10N]LsIA gains activity at human nAChRs. Molecular modelling of the extracellular binding domain of the α₃β₂ nAChR in conjunction with data from Everhart et al has revealed that a single amino acid situated just outside the C-loop may be responsible for the species specificity of certain α-conotoxins with activity at nAChRs. This finding is crucial when considering the potential development of these peptides for the treatment of diseases such as neuropathic pain.

Assessment of two-pore channels in MDA-MB-231 breast cancer cells.
Aisyah H Jahidin, Merril C Curry, Sarah J Roberts-Thomson, Gregory R Monteith. School of Pharmacy, The Univ of Queensland, Brisbane, QLD.

Introduction. Two-pore channels (TPCs) are calcium release channels localized to the endolysosomal system. The binding of the intracellular second messenger nicotinic acid adenine dinucleotide phosphate (NAADP) to TPC1 or TPC2 releases calcium from endolysosomal calcium stores (Ruas et al, 2010). This suggests possible roles for these channels in calcium-dependent processes, including cell death. TPCs in breast cancer have not yet been assessed, despite reports of altered calcium signaling in breast cancer cells via other calcium channels.

Aims. To evaluate the potential roles of TPC1 and TPC2 in MDA-MB-231 breast cancer cells.

Methods. TPC1 and TPC2 silencing was performed using ON-TARGETplus SMARTpool siRNAs and knockdown was confirmed using real time RT-PCR. MDA-MB-231 cells were treated with ABT-263, ionomycin or ceramide to induce cell death. Cells were stained with Hoechst 33342 and propidium iodide and then assessed using a high content imaging system (ImageXpress) to evaluate the consequences of TPC1 or TPC2 silencing on cell death.

Results. More than 75% knockdown was achieved for TPC1 or TPC2 in MDA-MB-231 cells. Silencing of TPC1 or TPC2 gene expression significantly attenuated ABT-induced cell death (3 and 10 uM ABT-263; \(P < 0.05\)). In contrast, ionomycin-induced cell death was significantly augmented by TPC1 or TPC2 silencing (3 uM Ionomycin; \(P < 0.05\)). However, TPC1 or TPC2 silencing did not alter ceramide-induced cell death at both submaximal (30 uM) and maximal (100 uM) concentrations.

Discussion. These data indicate that TPC1 and TPC2 may play a role in modulating cell death pathways. Future work is required to define the possible mechanisms involved.

**TRPV4 channels in basal-like breast cancer cells**

Siti Y N Jamaludin1, Felicity M Davis1, Amelia A Peters1, Thomas J Gonda1, Sarah J Roberts-Thomson1, Gregory R Monteith1. School of Pharmacy, The Univ of Queensland1, Brisbane, QLD.

Introduction. TRPV4 is a polymodal non-selective cation channel. TRPV4 is involved in processes including osmoregulation, mechanosensation and thermoregulation. Despite the established roles of other TRP channels in cancers (such as TRPV6), the role of TRPV4 in breast cancer has not been fully assessed.

Aims. 1) To assess TRPV4 mRNA levels in a panel of basal breast cancer cell lines; 2) to evaluate changes in TRPV4 mRNA levels with epithelial to mesenchymal transition (EMT) - a process implicated in cancer metastasis, in breast cancer cells; and 3) to assess the effects of activating and silencing TRPV4 on Ca2+ signalling in MDA-MB-468 breast cancer cells.

Methods. Real-time RT-PCR was used to assess TRPV4 mRNA levels in HCC1569, MDA-MB-468 and MDA-MB-231 basal-like breast cancer cells. Changes in TRPV4 levels associated with EMT were assessed using epidermal growth factor (EGF, 50 ng/mL) treatment for 48 h to induce EMT in MDA-MB-468 cells. Fluorescent imaging plate reader (FLIPR) Ca2+ assays were used to assess the effect of a TRPV4 pharmacological activator GSK1016790A (30 nM) in MDA-MB-468 cells. GSK1016790A-induced Ca2+ influx was assessed in MDA-MB-468 cells 72 h post-transfection with TRPV4 siRNA.

Results. TRPV4 mRNA levels were highest in MDA-MB-468 cells, followed by HCC1569 and MDA-MB-231 cells. Real-time RT-PCR analysis revealed no change in TRPV4 mRNA during EGF-induced EMT. Stimulation with GSK1016790A (30 nM) induced a robust and sustained Ca2+ influx in MDA-MB-468 cells. A dose-response curve of GSK1016790A in MDA-MB-468 cells showed an EC50 of approximately 3.9 nM. TRPV4 silencing in MDA-MB-468 cells decreased GSK1016790A-induced Ca2+ influx.

Discussion. MDA-MB-468 cells exhibit GSK1016790A-mediated Ca2+ influx that is sensitive to TRPV4 silencing. These results suggest that TRPV4-mediated Ca2+ influx is a feature of some breast cancer cells.

---

**A novel analytical approach reveals a distinct pattern of stimulus bias for an antipsychotic drug at the dopamine D2 receptor**

C Klein Herenbrink1, J Shonberg2, B Capuano2, A Christopoulos1, JR Lane1. Drug Discovery Biology1 and Medicinal Chemistry2, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia

Introduction. Most antipsychotics antagonize the dopamine D2 receptor (D2R) subtype as their main mechanism of action. Unfortunately, this antagonism also leads to extrapyramidal side effects. More current development of antipsychotics is focused on D2-selective partial agonists. Although several D2-selective partial agonists have been tested for the treatment of schizophrenia, only aripiprazole has made it to the market so far. It remains unclear why certain partial agonists are efficacious as a treatment for schizophrenia whereas others are not. One emerging hypothesis is that aripiprazole displays ‘stimulus bias’, that is, it promotes unique conformations of the D2R that signal selectively via certain signalling pathways and not others, and that this bias underlies its unique antipsychotic activity.

Aims. To determine if aripiprazole displays stimulus bias at the D2R and if it has a distinct pattern of bias from partial agonists with limited anti-psychotic efficacy such as S-3PPP.

Methods. The effect of various D2R ligands with known in vivo efficacy and their derivatives on both the inhibition of forskolin-induced cAMP production and the phosphorylation of ERK1/2 was determined using FlpIn CHO cells expressing the D2R. Data was analyzed using a method based upon the Black and Leff operational model of agonism to allow the quantification of stimulus bias (Kenakin et al., 2011).

Results. Aripiprazole is approximately 30-fold more biased towards the inhibition of cAMP production than towards ERK1/2 phosphorylation as compared to the typical D2R agonists dopamine or ropinirole and the partial agonist S-3PPP.

Discussion. Our novel analytical approaches have revealed that the antipsychotic aripiprazole displays a distinct pattern of stimulus bias as compared to the endogenous agonist dopamine or S-3PPP. This observation will provide a foundation for future studies aimed at relating in vitro activity with in vivo efficacy.

Development of a real-time, fluorescence based assay of mu-opioid receptor mediated inhibition of adenylate cyclase activity in Chinese hamster ovary cells
Alisa Knapman1, Mark Connor1. 1Aust. School of Adv. Med., Macquarie Univ., Sydney, NSW

Introduction. Activation of the mu-opioid receptor (MOR) leads to inhibition of adenylyl cyclase (AC) and reduction of cyclic adenosine monophosphate (cAMP) levels. Modulation of AC activity is frequently used as an assay for measuring opioid ligand potency and efficacy. Most currently used AC assays are single time point, require multiple reagents and/or cell lysis, with lengthy development times.

Aims. To develop a simple, fluorescence based assay of MOR-mediated AC inhibition in Chinese hamster ovary (CHO) cells.

Methods: CHOK1 cells stably expressing human MOR were grown in 96-well microplates. Membrane potential was measured using the Molecular Devices FLIPR membrane potential dye. Dye emission intensity increases on membrane depolarization and decreases upon hyperpolarization.

Results. Treatment of CHO cells with the AC activator forskolin (FSK) resulted in membrane hyperpolarization. Forskolin hyperpolarized cells with a pEC50 of 7.3±0.1 to a maximum of 52±2% from baseline. The hyperpolarization induced by FSK (300nM) was inhibited in a dose-dependent manner by the opioid agonists [D-Ala2,N-MePhe4,Gly-ol]-enkephalin (DAMGO, Emax 57±3%, pEC50 7.8±0.1), morphine (Emax 58±9%, pEC50 7.1±0.3) and buprenorphine (Emax 22±4%, pEC50 9.0±0.6). The effects of opioids were prevented by treatment with pertussis toxin (200ng/mL overnight), and blocked by naloxone (1ȝM). The FSK hyperpolarization was mimicked by Sp-8-CPT-cAMPS (100ȝM), a direct activator of protein kinase A. The FSK hyperpolarization was not blocked by K channel inhibitors tetr aethylammonium chloride (10mM), 4-aminopyridine (300ȝM), glibenclamide (10ȝM), VU-591 (100ȝM) or charybdotoxin (100nM). However, increasing extracellular K+ from 2.5mM to 30mM and 75mM decreased the FSK response from 52±2% to 26±2 % and 1±0.2%, respectively, implying the involvement of a K channel.

Discussion. This assay is a novel method for rapid, no-wash, real-time measurement of AC inhibition by MOR ligands in intact CHO cells, which may be suitable for high-throughput screening.

In vitro assessment of ligand-mediated μ-opioid receptor interaction with G protein and β-arrestin 2
Ai-Leen Lam1, Shannon O’Brien1, Marine Barral1, Nur-Syazwani A Rethwan1, Maree T Smith1,2. Centre for Integrated Preclinical Drug Development, and School of Pharmacy2, University of Queensland, Brisbane, QLD, Australia, 4072.

Introduction. Strong opioid analgesics such as morphine are the mainstay for the relief of moderate to severe nociceptive pain but they also produce adverse effects including respiratory depression, nausea, vomiting, sedation and constipation. Although most clinically used opioid analgesics produce analgesia through activation of the μ-opioid (MOP) receptor, there are between-opioid differences within individuals with respect to efficacy and tolerability. Recently, ligand-mediated preferential signalling at (μ) MOP, δ (DOP) and κ (KOP) receptors via their molecular transducers has been proposed to contribute to between-opioid differences within individuals in analgesia and tolerability observed in the clinical setting (Molinari et al, 2010).

Aims. To investigate the extent to which various opioid ligands promote coupling of the MOP receptor to β-arrestin 2 and G protein.

Methods. Bioluminescence Resonance Energy Transfer (BRET) was used to assess opioid-mediated coupling of the MOP receptor to either β-arrestin 2 or G protein. Human embryonic kidney 293 (HEK293) cells co-transfected with chimeric plasmids expressing Renilla luciferase-tagged opioid receptors with either green fluorescent protein-tagged β-arrestin 2 or the Gp_i subunit. Various opioid ligands were added to transfected cells, and the resulting BRET signals were measured using a luminometer.

Results. Opioid ligands exhibit differential agonist and antagonist signaling via β-arrestin 2 and G protein interactions at the MOP receptor.

Discussion. In vitro profiling of the extent to which opioid analgesics induce biased signaling via β-arrestin 2 and G protein-coupled pathways has the potential to enhance knowledge of signalling pathways that are linked to analgesia rather than adverse effects.

Taking advantage of kinetic data: an alternative approach to obtain affinity estimates from GPCR-mediated intracellular calcium mobilization.

Lauren T May1, Lloyd J Bridge1 & Stephen J Hill2. MIPS & Dept of Pharmacol, Monash University1, Parkville, VIC; School of Biomed Sci, Univ of Nottingham2, Nottingham, UK.

Introduction. G protein-coupled receptors (GPCRs) represent common therapeutic targets. Over the last decade, the primary high throughput-screening assays used for drug discovery have been calcium mobilization assays, which provide kinetic data at a resolution of 1-2 seconds. Despite the widespread use, the rapid and dynamic nature of calcium assays often introduce non-equilibrium artifacts upon the derivation of key pharmacological parameters for drug discovery, including antagonist equilibrium dissociation constants (Charlton & Vauquelin, 2010).

Aim. To investigate the influence of agonist and antagonist exposure time on human M3 muscarinic acetylcholine receptor (M3 mAChR) mediated calcium mobilization.

Methods. CHO cells stably expressing the human M3 mAChR (M3-CHO) were incubated in loading buffer containing Fluo-4AM. Cells were then washed and agonist-mediated changes in fluorescence measured every 1.52 seconds in the absence or presence of antagonist pre-treatment (30 minutes) or simultaneous addition.

Results. In M3-CHO cells, the inhibition of agonist- (oxotremorine-M, carbachol, pilocarpine and bethanecol) mediated calcium mobilization by atropine (100 nM), NMS (10 nM) clidinium (100 nM) and ipratropium (100 nM) varied with both agonist and antagonist exposure time. However as a function of time, the change in agonist potency in the presence of antagonist was agonist independent and reached a common plateau that correlated well with previous equilibrium estimates of corresponding antagonist equilibrium dissociation constants.

Discussion. Kinetic analysis provides an alternative method to define antagonist equilibrium dissociation constants from non-equilibrium calcium mobilization data. Employing a kinetic analysis can generate robust affinity estimates and as such assist GPCR drug discovery programs.


The efficacy of kappa-opioid receptor agonists in pain and inflammation

Michael Morgan1, Aaron Heffernan1, Amitha Hewavitharana1, Paul N. Shaw1, Peter J. Cabot1, School of Pharmacy, The University of Queensland, Brisbane, Queensland1.

Introduction. The κ-opioid receptor (KOR) agonists have received increasing interest for the treatment of pain, due to their reduced side effect profile, reduced addiction and anti-inflammatory properties.

Aims. This study aimed to examine the endogenous KOR agonist Dynorphin 1-17 and the synthetic KOR agonist U50488H efficacy at the KOR receptor, in a unilateral model of pain and anti-inflammatory properties.

Methods. HEK-KOP cells were stimulated to produce cAMP with L-858501 (300 nM), followed by treatment with increasing concentrations of the KOR agonists. Intracellular cAMP levels measured by alphascreen. Male Wistar rats paws were unilaterally treated with FCA (150 μL). After 4 days the inflamed paw was administered agonists (500 μM, 50μL/paw) and paw withdrawal measured with an analgesiometer. A monocyte cell line (THP-1) were differentiated to macrophages with PMA (50nM) for 48h, and then treated with KOR agonists at increasing concentrations for 2 h, followed by LPS (1μg/ml). Media was collected after 4 h and analysed for IL-1β by alphasila.

Results. U50488H and Dynorphin 1-17 inhibited cAMP production, with an IC50 of 2.7nM and 0.2nM respectively. U50488H resulted in a significantly increased paw withdrawal threshold in the inflamed paw, but this was not seen with equimolar concentrations of Dynorphin 1-17. Both U50488H and Dynorphin 1-17 produced a concentration dependent inhibition of IL-1β release from THP-1 cells.

Discussion. U50488H and Dynorphin 1-17 potently activate the KOR receptor, but only U50488H inhibited nociception in the inflamed paw. This is likely due to increased metabolism of Dynorphin 1-17 as we described previously for inflamed tissue. The dose-dependent inhibition of TNF-α and IL-1 with U50488H has previously been described, and this study further substantiates those findings.
Anti-inflammatory kinase inhibitors attenuate invasiveness and chemoresistance of glioblastoma cells
Yiu T Yeung1, Melissa Tang2, Ruiwen Heng3, Michael Buckland4, Gilles Guillemin3, Thomas Grewal1, Lenka Munoz2
Faculty of Pharmacy, University of Sydney, NSW1; Discipline of Pharmacology, School of Medical Sciences, University of Sydney, NSW2; Department of Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, NSW3; Discipline of Pathology, School of Medical Sciences, University of Sydney, NSW4

Introduction. Glioblastoma (GBM) is a fatal brain tumour. An important factor that drives the invasion of transformed cells, including human glioblastoma, is the development of an inflammatory microenvironment. Chronic inflammation in combination with infiltrated macrophages and oncogenic mutations contribute to up-regulate tumour-promoting cytokines which support migration, invasiveness and chemotherapy resistance in carcinogenesis. p38 mitogen-activated protein kinase (MAPK) plays a critical role in the development and amplification of inflammation accompanying various CNS disorders. However, it is yet unclear whether elevated inflammatory cytokine secretion and p38 MAPK activity are potential risk factors for the expansion of GBMs.

Aim: To determine the function of p38 MAPK signaling pathway in GBM pathophysiology.

Methods: p38 MAPK inhibitors were tested in multiple inflammation, migration, invasion and proliferation assays employing primary human microglia and GBM cells. We also tested p38 MAPK inhibitors in glioblastoma cells carrying epidermal growth factor receptor vIII mutation (EGFRvIII) as approximately 40-50% of glioblastomas are characterized by EGFR gene amplification and mutation; all of which are implicated in tumour growth, invasion and poor response to therapy.

Results: Inhibition of p38 MAPK pathway efficiently reduced the formation of an inflammatory glioblastoma microenvironment and attenuated aggressive phenotype of GBM cells. Investigation of the molecular mechanism revealed that inhibition of the p38 MAPK downstream kinase MK2 and regulation of HuR nuclear-cytoplasm shuttling are responsible for the anti-inflammatory activity. Importantly, anti-inflammatory kinase inhibitors significantly restored chemosensitivity of GBM cells to temozolomide, the standard chemotherapeutic used in GBM therapy.

Discussion: Together, our data suggest that regulation of inflammatory GBM microenvironment with anti-inflammatory kinase inhibitors may represent a useful approach to improve the current management of this serious disease. Development of follow-up kinase inhibitors and investigation of MK2 in human GBM specimens will also be discussed.


Assessing the cytotoxicity of natural products on squamous cell carcinoma and human keratinocyte cell lines
Thao T Nguyen, Amitha K Hewavitharana, Marie-Odile Parat, Paul N Shaw, School of Pharmacy, The University of Queensland, Brisbane, QUEENSLAND

Introduction. In-vitro cell culture studies are valuable tools for screening chemo-preventive potential of natural products, which is receiving increasing attention due to continuing increase in new cancer cases as well as cancer deaths, and the cost and side effects of current radiation or chemotherapy. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay quantifies viable cells in proliferation and cytotoxicity studies. Although the method is rapid and convenient, various parameters have been identified that can result in an under- or over-estimation of the tested drugs. Therefore, it is essential to refine the assay parameters for tested cell lines in order to have accurate and reliable results.

Aims. To establish parameters for cytotoxicity studies of natural products using MTT assay on cancerous and non-cancerous skin epithelial cells.

Methods. We used squamous cell carcinoma (SCC25) and non-cancerous human keratinocyte (HaCaT) cell lines and MTT assay with 595-nm reading in a 96-well plate format. We optimised cell seeding densities, serum content of media; determined the maximum solvent concentration and identified a suitable positive control for the assay.

Results. The optimised method required different seeding cell numbers for two cell lines and different serum concentrations for the duration of the assay in order to reach interpretable optical densities (0.4-0.8) at the end of experiment. With the objective of testing natural extracts prepared in aqueous ethanol, we determined that the optimal, final concentration of ethanol in media is 0.3%. Epigallocatechin-3-gallate (EGCG) was used as positive control and exhibited significantly more toxicity in SCC25 than in HaCaT. Control wells with medium only added with EGCG revealed the absence of intrinsic reducing activity of EGCG in our optimised assay.

Discussion. The method that we have refined can be applied to screening extracts from natural products selectively to identify candidates with anti-proliferative effects on SCC25 cancer but not on HaCaT non-cancer cell line.
Alteration of SKBR3 cancer cell proliferation by silencing specific calcium pumps, channels and channel modulators.

Elena Pera¹, Amelia A. Peters¹, Sarah J. Roberts-Thomson¹, Gregory R. Monteith¹. School of Pharmacy, The Univ of Queensland¹, Brisbane, QLD.

Introduction. HER2-positive breast cancers are characterised by an overexpression of the growth factor receptor HER2 and represent about 20% of all breast cancers. Calcium transporters and modulators have been studied in several types of cancers, including breast cancer and their altered expression may contribute to the development and progression of some breast tumours. However, the expression and the effect of silencing of calcium transporters and modulators have not been fully evaluated in HER2-positive cells, such as SKBR3 cells.

Aims. To evaluate the effect of silencing of calcium transporters and modulators on the proliferation of SKBR3 cells.

Methods. Dharmacon siRNA was used to individually silence 16 calcium pumps, channels and channel modulators in SKBR3 cells. The effect on cell proliferation (144 h post siRNA) was evaluated using EdU-Alexa Fluor® 555 staining (Life Technologies) and high content imaging. Real time RT-PCR was used to confirm the silencing of the transporters and to evaluate their expression level.

Results. In SKBR3 cells there are greater levels of the stromal interaction molecule 1 (STIM1) mRNA compared to its related isoform STIM2; whereas two pore channel (TPC2) mRNA levels are lower compared to TPC1. Silencing of STIM1 and TPC2 significantly decreased the proliferation of SKBR3 cells. Calcium transporters such as plasma membrane calcium ATPase (PMCA) isoforms 1 and 4, secretory pathway calcium-ATPase (SPCA) isoforms 1 and 2 and the transient receptor potential (TRP) cation channel TRPV6 showed relatively high mRNA levels in SKBR3 cells, but silencing of these channels and pumps did not affect the proliferation of SKBR3 cells.

Discussion. These studies suggest that the proliferation of SKBR3 breast cancer cells is decreased by STIM1 and TPC2 silencing. Thus, STIM1 and TPC2 should be the focus of further investigation in HER2-positive breast cancers.

Immunohistochemical analysis of PMCA2 expression in normal and malignant human breast tissues

Amelia A Peters¹, Wei C Lee¹, Chanel E Smart², Lynne Reid², Leonard da Silva², Sunil R Lakhani², Sarah J Roberts-Thomson¹, Gregory R Monteith¹. School of Pharmacy, The Univ of Queensland¹, Brisbane, QLD; The UQ Centre for Clinical Research (UQCCR), The Univ of Queensland², Brisbane, QLD.

Introduction. PMCA2 is an isoform of the plasma membrane Ca²⁺ ATPase that pumps Ca²⁺ from the cytosol into the extracellular space. Expression of PMCA2 is significantly increased in mouse mammary glands during lactation where it plays a major role in the excretion of Ca²⁺ into milk; however PMCA2 expression has not been assessed in human mammary glands during lactation. In breast cancer cells PMCA2 over-expression reduces apoptosis, but its assessment in breast cancer is still limited.

Aims. To compare PMCA2 expression in normal breast tissue and breast tissue exhibiting lactational change and; to assess PMCA2 expression in human malignant breast samples.

Methods. Formalin-fixed, paraffin-embedded samples of non-cancerous and malignant breast tissues assembled into tissue microarrays were assessed for PMCA2 by immunohistochemistry using a rabbit anti-PMCA2 ATPase polyclonal antibody. Samples which showed membranous staining were considered PMCA2 positive.

Results. Membranous PMCA2 expression was observed in luminal epithelium of breast tissue exhibiting lactational change. PMCA2 expression was observed in 9 of 96 breast tumours (9.4%). These preliminary studies suggest that there is no obvious significant correlation with estrogen, progesterone or HER2 receptor status. Our results also identify that PMCA2 expression is not lost during lymph node metastasis.

Discussion. The findings indicate that PMCA2 is up regulated in human lactation and is a feature of some breast cancers. Inhibitors of PMCA2 may represent a therapeutic strategy for women with breast cancers that overexpress this Ca²⁺ pump.
Prediction of the GAG-binding interactions of four-helical cytokines
Maryam S. Masoum1, Neha S. Gandhi2, Mark Agostino2 and Ricardo L. Mancera1,2School of Pharmacy, Curtin university1,Perth, WA; School of Biomedical Sciences, Curtin University,Perth, WA.

Cytokines are regulators of intracellular communication that orchestrate virtually every response to infection, injury and inflammation. The interactions of cytokines with cell surface carbohydrates known as glycosaminoglycans (GAGs) play a pivotal role in the activation of cytokine-mediated biological responses. While an increasing number of cytokines have been shown to bind specifically to GAGs, little is known about the structural specificities of the underlying interactions. Understanding the GAG-binding properties of cytokines is ultimately the cytokine activation process and realizing its therapeutic potential. Here we report a combined approach using the analysis of evolutionary amino acid conservation, the computation of electrostatic potential molecular surfaces and molecular docking simulations of representative small heparin fragments, all of which have been used to predict potential GAG-binding sites on helical cytokines. Specific amino acid residues forming potential heparin-binding sites were identified for several cytokines, including interleukin-6 (IL-6), ciliary neurotrophic factor (CNTF), granulocyte colony stimulating factor (G-CSF), prolactin (PRL), erythropoietin (EPO), thrombopoietin (TPO) and granulocyte colony stimulating factor (GM-CSF). Three basic amino acids (arginine (Arg), lysine (Lys) and, to a lesser extent histidine (His)) were found to constitute the GAG binding sites and mediate the interactions with small heparin fragments, with the predicted free energies of binding ranging from -1 to -10 kcal/mol.

Investigating the mechanism of RXFP1 activation
Brad L Hoare1,2, Sharon Layfield2, Daniel J. Scott2, Ross A.D. Bathgate2.
1Department of Pharmacology, The University of Melbourne, Parkville, Victoria.
2The Florey Institute of Neuroscience and Mental Health, Parkville, Victoria

Introduction. The cognate receptor for relaxin is RXFP1, a G protein coupled receptor (GPCR) containing a large extracellular domain composed of a leucine rich repeat (LRR) region and an N-terminal low density lipoprotein receptor type A (LDLa) module. The current hypothesis of ligand mediated RXFP1 activation is that a receptor homodimer is formed, with one receptor binding relaxin and in turn activating the second receptor in the dimer in a transactivation manner, possibly via the LDLa module.

Aims. To test this hypothesis, co-expression of two different inactive RXFP1 mutant receptors, binding deficient (BD) and signalling deficient (SD), was performed to test if such co-expression could reconstitute normal relaxin-stimulated cellular responses, hence indicating transactivation.

Methods. Flow-cytometry was used to measure cell surface expression of transiently transfected mutant receptors in HEK293T cells, and this technique was adapted for FACS selection of cell lines that highly express these receptors. Inactive BD1 receptor (RXFP1-E277Q/D279N) was transfected into the cell lines highly expressing the semi-active SD1 (RXFP1-D451N) or SD3 (RXFP1-Y511G/Y599A) receptors, and relaxin-stimulated cellular responses were measured using plate-based CRE reporter gene assays as well as direct cAMP measurements.

Results. As expected, stimulation of BD1 or SD1 receptors alone with relaxin did not result in cAMP accumulation. The co-expression of BD1 and SD receptor variants did not reconstitute normal relaxin-stimulated cellular responses, however there was a small decrease in the efficacy of relaxin-stimulated cellular responses for semi-active SD variants when co-expressed with BD1.

Discussion. No clear evidence of RXFP1 transactivation was observed in these studies, however it is possible that the decrease in efficacy of relaxin on partially active SD variants indicated a dominant-negative effect that would support the transactivation hypothesis. There are many alternative mechanisms by which transactivation may be occurring, and the SD/BD model may have been inadequate to show this.
Medication safety issues in older Australians: results from a national medicines census
Joanne Barnes1, Tessa K Morgan2, Margaret Williamson3, Jared Brown2, Michelle Sweidan4, Marie Pirotta5, Kay Stewart6, School of Pharmacy, Univ of Auckland1, Auckland, NZ; National Prescribing Service2, Sydney, NSW; Dept of General Practice, Univ of Melbourne3, Melbourne, VIC; Centre for Medicine Use and Safety, Monash Univ4, Melbourne, VIC; (introduced by Lynne Bye, Univ of Auckland, Auckland, NZ).

Introduction. The use of medicines, including complementary medicines (CMs), for the prevention/treatment of disease is common. Information is limited, however, on patterns of medicines use, including concurrent use of multiple medicines, prevalence and types of medication safety issues, and users’ reported responses to such issues.

Aims. To examine: the prevalence of concurrent use of medicines; prevalence and types of medication safety issues; self-reported response to medicines-related problems in Australians aged ≥50 years.

Methods. Cross-sectional study involving a questionnaire posted to a random sample of 4500 Australians aged ≥50 years, selected from the Australian electoral roll, between June 2009 and February 2010. Data were collected, using a pre-piloted 24-hour medicines diary, on self-reported medicines’ use and experiences of suspected ADRs (sADRs). A potential drug interactions (PDIs) list was developed from scientific literature and reviewed by pharmacists.

Results. Response rate: 37.3%. A majority of respondents (87.1%, 95%CI:85.3-88.6%) had taken one or more medicines in the previous 24 hours. Of these, 5.5% were exposed to a PDI involving conventional medicines only, and 12.3% a PDI involving a medicines combination (including CMs). Of all respondents, 43.3% (95%CI:41.0-45.8%) had used ≥5 medicines and 10.7% (95%CI:9.3-12.3%) ≥10 medicines in the previous 24 hours; of these, 8.3% and 20.1%, respectively, were exposed to a PDI involving conventional medicines only. Overall, 18.4% (95%CI:16.2%-20.8%) of medicines users experienced sADRs in the previous year. In response, 76.4% of these informed their doctor about their last sADR and 38.9% stopped taking medicines.

Discussion. The prevalence of use of medicines, including concurrent use of multiple medicines, among Australians aged ≥50 years is high. A substantial proportion experiences sADRs and/or is exposed to potentially interacting medicines. These findings emphasise the need for open communication between health professionals and patients about all medicines used, including CMs, and for vigilance regarding ADRs, including drug interactions.

Study of natural health product adverse reactions (SONAR): active surveillance of adverse events following concurrent natural health product and prescription medicine use in community pharmacies
Joanne Barnes1, Candace Necyk2, Heather Boon3, Brian C Foster4,5, Ross T Tsuyuki1,6,7, John T Arnason8, Sunita Vohra2,6,7,9 for the SONAR group. School of Pharmacy, Univ of Auckland1, Auckland, NZ; Dept of Pediatrics, Univ of Alberta2, Edmonton, ALBERTA; Leslie Dan Faculty of Pharmacy, Univ of Toronto3, Toronto, ONTARIO; Faculty of Medicine, Univ of Ottawa4, Ottawa, ONTARIO; Health Canada5, Ottawa, ONTARIO; School of Public Health, Univ of Alberta6, Edmonton, ALBERTA; Faculty of Medicine and Dentistry, Univ of Alberta7, Edmonton, ALBERTA; Dept of Biology, Univ of Ottawa8, Ottawa, ONTARIO; Women and Childrens’ Health Research Institute9, Edmonton, ALBERTA. (introduced by Lynne Bye, Univ of Auckland, Auckland, NZ).

Introduction. Many consumers use natural health products (NHPs) concurrently with prescription medicines. As NHP-related harms are under-reported through passive surveillance, the safety of concurrent NHP-prescription medicine use remains unknown.

Aims. To conduct active surveillance in participating community pharmacies to identify adverse events following concurrent NHP-prescription medicine use.

Methods. Participating pharmacy staff from ten community pharmacies in the Greater Toronto Area, Ontario, Canada, asked individuals collecting prescription medicines about (i) concurrent NHP-prescription medicine use in the previous three months and (ii) experiences of adverse events. If an adverse event was identified and if the patient provided written consent, a research pharmacist conducted a guided telephone interview to gather further, detailed information after also obtaining (and documenting receipt of) verbal consent. A study committee, comprising an NHP expert, a clinical expert, and a clinician with expertise in pharmacology and NHPs, assessed AE reports using accepted algorithms and the World Health Organization (WHO) causality assessment criteria to determine: (i) the likelihood of a causal relationship; (ii) the likelihood of an NHP-drug interaction; (iii) the need for pharmaceutical analysis of NHP(s) concerned.

Results. Over a total of 112 pharmacy weeks, 2615 patients were screened, of whom 1037 (39.7%; 95%CI:37.8-41.5%) reported concurrent NHP and prescription medicine use. A total of 77 patients reported a possible AE (2.94%; 95%CI: 2.4-3.7%), which represents 7.4% of those using NHPs and prescription medicines concurrently (95%CI: 6.0-9.2%). Of 15 patients available for an interview, 4 (26.7%; 95%CI: 4.3- 49.0%) reported an AE that was determined to be “probably” due to NHP use, including concurrent use with prescription medicines.

Discussion. Although not without challenges, active surveillance of NHPs, particularly where used concurrently with prescription medicines, in the community pharmacy setting is feasible and can generate adverse event data of sufficient quality to allow causality assessment of potential harms.
**The impact of medications on older people with falls**

Sarah N Hilmer¹,², Danijela Gnjidic¹,²,⁴, Mark J Gillett³, Peter R Carroll²,⁵, Slade T Matthews²,⁵ & Alexander N Bennett¹,³ Dep of Clin Pharmacol, Royal North Shore Hosp¹, St Leonards, NSW; Sydney Medical School, Univ of Sydney ², Sydney, NSW; Emergency Dep, Royal North Shore Hosp ³, St Leonards, NSW; Faculty of Pharmacy, Univ of Sydney ⁴, Sydney NSW; Dep of pharmacology, Univ of Sydney⁵, Sydney NSW.

Introduction. Medication exposure, defined as polypharmacy (≥5 medications) or exposure to Falls Risk Increasing Drugs (FRIDs, cardiovascular or psychoactive medicines) has been associated with poor outcomes in older adults with falls. However, there is limited evidence in relation to medication use including drug-drug interactions (DDIs) and clinically important drug-disease interactions (DDSI), and adverse outcomes in frail adults with falls.

Aims. To investigate medication exposures (polypharmacy, FRIDs, DDIs and DDSI) and falls in robust and frail hospitalised patients.

Methods. Patients aged over 60 years admitted with a fall to Royal North Shore Hospital (RNSH) were recruited. Clinical and medication data was collected on admission, discharge and at 2 months. Adverse outcomes were defined as rehospitalisation, falls, institutionalisation, and death.

Results. 129 patients were recruited (mean age 81.5 ± 7.8) with 59 robust and 70 frail. On admission, frail patients had significantly higher rates of polypharmacy (frail 68%, p=0.001), DDSIs (54%, p=0.002), DDIs (36%, p=0.001) and mean (+/-SD) FRID number (4±2, p=0.01) compared to the robust. Between admission and discharge, no significant change was seen in medication exposure. At 2 months, FRIDs were significantly correlated with adverse outcomes (1.8; 1.2-2.6). Adjusted ORs revealed no significant relationship between outcomes and polypharmacy (1.5; 0.5-4.3), DDIs (1.0; 0.8-1.4) or DDSIs (2.9; 0.95-8.9).

Discussion: Frail patients had higher medication exposure compared to the robust. FRID use was significantly correlated with adverse outcomes.

**An essential medicines list for children: Are they still therapeutic orphans?**

Noel Cranswick¹, Paul Gallagher¹, Sean Beggs². Clinical Pharmacology, Royal Children’s Hospital¹, Parkville, VICTORIA; Paediatrics and Child Health, University of Tasmania², Hobart, TASMANIA.

Introduction. The Essential Medicines List (EML) is produced biennially by the World Health Organisation (WHO). The minimum medicine needs for a basic health care system are listed, including the most efficacious, safe and cost-effective medicines for priority conditions. From its inception in 1997, the list had a focus on Adult medicines although there were some paediatric medicines listed. Goal 4 of the millennium development goals aimed to reduce child mortality; hence, there was a need to focus on medicines for children.

Aims. The aims were to assess the current state of the EML in regards to children; implement a policy of increasing paediatric medicines on the list and then reassess whether the policy had been effective.

Results. After assessing the 2003 EML, the WHO requested that we assess the 2005 list in which we identified 77 medicines on the EML relevant to children but with no paediatric formulation (PF) listed. After a series of meetings, the WHO established a subcommittee of the EML specifically for children’s medicines as well as a children’s EML (EMLc) in 2007. The subcommittee recommended medicines for children be added to the EML; these needing to be ratified by the full EML committee before addition to the EMLc. After 3 iterations of the list by 2011 some progress had been made with 23 medicines for children being added since the original assessment and over 310 medicines listed on the new EMLc (see Table).

Discussion. While there has been a significant improvement with the availability of EMLc, more formulations development is needed for children’s medicines.

The ABCD of Clinical Pharmacokinetics
Matthew P Doogue1,2, Thomas M Polasek1. Discipline of Clinical Pharmacology, Flinders University School of Medicine1, Adelaide, SA; Department of Clinical Pharmacology, Flinders Medical Centre2, Adelaide, SA.

Introduction. ADME is the acronym for absorption, distribution, metabolism and excretion that has described pharmacokinetics for 50 years.

Aims. To review the historical origin of ADME; to critically appraise current uses of ADME; and to present a new acronym for teaching clinical pharmacokinetics.

Discussion. ADME was first proposed by Nelson in 1961, rephrasing resorption, distribution, consumption and elimination used by Teorell in 1937. Drug: entering the body (A), moving about the body (D), changing within the body (M), and leaving the body (E). Over time the use of ADME has diversified according to the needs of the user, in particular for mechanisms: crossing the gut wall (A), movement between compartments (D), mechanisms of metabolism (M), excretion or elimination (E), and transport (T) is sometimes added. Variable use of ADME often causes confusion.

In teaching pharmacokinetic principles we follow the active drug moiety through the body in space and time using the schematic shown. We use the acronym ABCD, standing for administration, bioavailability, clearance and distribution. Administration is factors relating to dosing and adherence. Bioavailability is the active drug moiety arriving in the systemic circulation (Nelson’s A). Clearance is the active drug leaving the systemic circulation (ME). Distribution is to the site/s of action (D).

Conclusion. ADME is an old friend that has served pharmacokinetics well, but it has quirks that sometimes make teaching pharmacokinetic principles difficult. Seventy-five years after Teorell, ABCD is an alternative acronym for teaching clinical pharmacokinetics.

A population pharmacokinetic (popPK) model to describe the renal clearance and the effect of transporter genetic variants on the pharmacokinetics of metformin in patients with type 2 diabetes mellitus (T2DM).
Janna K Duong1,2, Shaun S Kumar1,2, Carl M Kirkpatrick1, Anna Lindstrom2, Garry G Graham1,2, Kenneth M Williams1,2, Richard O Day1,2. School of Medical Sciences, Univ of New South Wales1, Kensington, NSW; Dept of Clin Pharmacol, St Vincent’s Hospital2, Darlinghurst, NSW; Centre for Medicine Use and Safety, Monash University3, Parkville, VIC.

Introduction. Metformin is used in the treatment of T2DM despite large between-subject variability (BSV) in the PK. An effect of SNPs (OCT1, OCT2, OCT3, MATE1 and PMAT) on the apparent clearance (CL/F) of metformin has been reported, however, an effect on the renal clearance of metformin (CLR) has not been investigated in patients with T2DM.

Aims. To investigate CLR and effect of SNPs on CLR in patients with T2DM.

Methods. Patients with T2DM (n=21) were recruited. Intensive blood sampling (8–10 samples) and urinary output was collected in 16 patients over a dosage interval (for immediate-release, IR, 12 h; extended-release, XR, 24 h). Sparse samples were collected from the remaining 5 patients (8 blood samples, 6 weeks) with the collection of timed (2-5 h) urine samples. A 2-compartment model was developed using NONMEM with parameters for apparent non-renal clearance (CLR/F), renal clearance (CLR), oral availability (F), first-order absorption (ka) for IR and zero-order absorption for XR. Effects of F on IR and XR doses were evaluated. Age, body weight, lean body weight, creatinine clearance (CLCR) and genotype (52 SNPs), were evaluated as covariates of the PK parameters.

Results. The mean and BSV of the PK parameters included: CLR/F, 24.6 L/h (57%); CLR, 27.3 L/h (19%); Vc/F, 180 L (45%); F, 1 (57%). The fractional elimination via the renal route ranged from 11% to 80%. Only the measured urinary CLCR was a significant covariate for CLR. None of the SNPs was a significant covariate for CLR.

Discussion. It would appear that the large variability in metformin PK is due to variable oral availability and variable excretion of metformin via the renal route. None of the SNPs were significant covariates for CLR. More patient data are required to detect the covariate-effect of variant transporters.
**Population pharmacokinetics of Factor VIII in paediatric patients.**

Kirsten LB Jensen¹, Noel E Cranwick², Chris Barnes³, James Ziogas⁴. Dept of Pharmacol, Univ of Melbourne¹, Parkville, VIC; Dept of Clinical Pharmacol, Royal Childrens Hosp², Parkville, VIC; Dept of Haematology, Royal Childrens Hosp³.

Introduction. The pharmacokinetics of Factor VIII (FVIII) in children with haemophilia A (HA) has been poorly characterised due to the requirement of intensive blood sampling in conventional studies. Despite this, knowledge of individual FVIII pharmacokinetics (PK) in prophylaxis is critical for maintaining a trough level of FVIII for prevention of haemarthrosis in haemophilic patients.

Aims. To produce a population pharmacokinetic model of FVIII in children based on a sparse sampling schedule, and to use this model to determine optimal doses in an individually-tailored fashion.

Methods. Sparse FVIII concentration data from four patients with HA at the Royal Children’s Hospital was combined with rich data from 20 paediatric patients in a previous FVIII PK study, and analysed using the population PK software, NONMEM.

Results. Body weight, baseline von Willebrand’s Factor (VWF), and baseline FVIII inhibitor level were all identified as patient characteristics significantly affecting FVIII CL or V. Doses calculated based on NONMEM estimates for the new RCH patients were able to be minimised, to a 43.75 ± 15.73 % (n=, p<?) reduction in annual FVIII use.

Discussion. The identification of VWF as a significant covariate of FVIII clearance is a novel finding that may explain to a large degree variations in FVIII PK previously unexplained by other population PK studies, and will have implications in the treatment of patients with haemophilia A. The minimisation of FVIII dosage in these four patients is a promising starting point for increased cost-effectiveness in the use of an expensive treatment.

---

**The pharmacokinetics of paracetamol and its metabolites: A population approach to investigating hepatic intrinsic clearance in old age.**

Claire Johnston¹,², Andrew J McLachlan³,⁴, Carl M Kirkpatrick⁵,⁶ & Sarah N Hilmer¹,². Sydney Medical School, University of Sydney¹, Sydney, NSW; Dept of Clinical Pharmacology and Aged Care, Royal North Shore Hospital³, St Leonards, NSW; Centre for Education and Research on Ageing, Concord RG Hospital⁵, Concord, NSW; Faculty of Pharmacy, University of Sydney⁶, Sydney, NSW; Centre for Medicine Use and Safety, Monash University⁵, Melbourne, Victoria.

Introduction. Paracetamol can be used as a marker of intrinsic clearance by the liver. Investigating its pharmacokinetics in older people will provide important information about liver function in older age, which may be applied to other drugs metabolized in similar ways.

Aims. The aim of this study was to determine the impact of frailty on the pharmacokinetics of paracetamol and its metabolites in older inpatients.

Methods. Data from two studies were pooled; one conducted in healthy volunteers with intensive plasma sampling and the other an observational study of inpatients over 70 years old, who had sparse samples taken. Both the paracetamol glucuronide and sulfide metabolites were measured for each sample along with the parent drug concentration using HPLC. Population pharmacokinetic analysis was undertaken using NONMEM (version 7.2).

Results. The total study population was 219; 20 healthy volunteers and 199 inpatients. The average age of the volunteers was 35.7 years and the inpatients was 84.7 years. There were 139 frail patients and 61 non-frail. The best model was a one-compartment linear pharmacokinetic oral model with interindividual variability on all parameters. There was high variability in both populations.

Discussion. Frailty will be used to investigate the variability in the pharmacokinetics of paracetamol in the older patients. Decreasing variability in the model will allow for more predictable therapeutic outcomes in older people.
Baseline plasma urate: the only determinant for dosage selection of allopurinol.
Diluk RW Kannangara1,2, Garry G Graham1,2, Sophie L Stocker1,3, Ian Portek4, Kevin D Pile5, Praveen L Indraratna1,2, Indira Datta1,2, Kenneth M Williams1,2, Richard O Day1,2. Dept of Clin Pharmacol, St Vincent’s Hosp1, Darlinghurst, NSW; School of Medical Sciences, Univ of New South Wales2, Kensington NSW; Faculty of Pharmacy, Univ of Sydney3, Sydney, NSW; Dept of Rheumatol, St George Hosp5, Kogarah, NSW; Dept of Medicine, Campbelltown Hosp3, Campbelltown, NSW.

Introduction. Allopurinol is the most common treatment for gout and acts by lowering urate synthesis through xanthine-oxidoreductase inhibition. Many patients continue to experience acute gout attacks despite, apparently, adhering to allopurinol therapy. Lower doses are recommended in patients with impaired renal function but this approach often fails to lower plasma urate concentrations sufficiently.

Aim. To predict the optimum dosage of allopurinol in patients with gout.

Methods. Gouty patients (n=46) were treated with increasing doses of allopurinol ranging from 50-600 mg/day. Plasma urate and creatinine concentrations were measured at baseline and during treatment. The plasma concentrations of oxypurinol were measured in 26 of these patients. All urate concentrations were fitted to a dose-response model (Figure 1) by non-linear regression (R software version 2.15.0).

Results. Plasma concentrations of urate during treatment of allopurinol (Uₜ) were highly dependent on the dose of allopurinol (D) and the baseline plasma urate concentration (Uₚ). The best-fit estimates (mean, 95% CI) of the dose that inhibits 50% of urate concentrations (ID₅₀) and the apparent resistant plasma concentration of urate (Uₐ) were 226 mg (167-303) and 0.20 mmol/L (0.14-0.25), respectively. The addition of creatinine clearance (CLCR, normalised to 120 mL/min) as a covariate did not improve the fit (P=0.09). In the subset of 26 patients, replacement of D with oxypurinol concentrations did not improve the fit (residual sum of squares = 0.18).

Discussion. Higher baseline plasma urate concentrations require higher doses in order to reach sufficiently low urate concentrations. The determinant of the final dosing rate of allopurinol is the baseline plasma urate concentration and is independent of renal function.

Optimising ribavirin therapy for hepatitis C patients.
Ashmit Kaur1,2, John E Ray1, Kenneth M Williams1,2, Garry G Graham1,2, Shaun S Kumar1,2, Brian Egan1, Gail Matthews3, Richard O Day1,2. Dept of Clin Pharmacol, St Vincent’s Hosp1, Darlinghurst, NSW; School of Medical Sciences, Univ of New South Wales2, Kensington, NSW; Dept of Immunol B Ambul Care, St. Vincent’s Hosp3, Darlinghurst, NSW.

Introduction. Hepatitis C Virus (HCV) is treated with a combination therapy of ribavirin and pegylated-interferon. Increasing concentrations of ribavirin increase sustained virological response (SVR) rates while also increasing the risk of haemolytic anaemia. Hence, therapeutic drug monitoring has been recommended to individualise dosing regimens and achieve therapeutic drug concentrations.

Aim. To construct and validate a Bayesian model to predict day 28 plasma concentrations of ribavirin from either day 7 or day 14 plasma concentrations of ribavirin.

Methods. A population pharmacokinetic model was constructed (Kinetica version 5.0) using clinical data from Caucasian and Japanese populations. The model parameters were incorporated into the Bayesian forecasting software, Abbottbase. Plasma ribavirin concentrations were measured by HPLC in HCV patients (n=10) treated with ribavirin and pegylated-interferon at day 0, 7, 14 and 28 of therapy and were entered into Abbottbase. The day 28 AUC (observed) was then calculated. Trough and 2 h post-dose concentrations for day 7 or day 14 (n=10), were used to predict the AUC on day 28. Bias and precision were calculated for observed versus predicted AUC.

Results. A two-compartment population pharmacokinetic model provided the best fit to the ribavirin plasma concentrations. The mean (CV%) CL, V1 and V2 were 7.2 L/h (31%), 380 L (31%) 5917 L (40%), respectively. The observed versus predicted AUC at day 28 indicated a bias and precision of 3.3% and 15.7% from the data at day 7. Bias and precision were 2.2 % and 12.3% from the data at day 14.

Discussion. The bias and precision indicate that the constructed model is sufficiently accurate to predict dose regimens from plasma concentrations taken on day 7 or 14. Application of this approach should result in early dose adjustment which may improve SVR rates while minimizing toxicity.
Dosage adjustment of medications in patients with renal impairment: how consistent are drug information sources?
Aarati Khanal\textsuperscript{1}, Ronald L Castelino\textsuperscript{1}, Gregory M Peterson\textsuperscript{1}, Matthew Jose\textsuperscript{2}. School of Pharmacy, University of Tasmania\textsuperscript{1}, Hobart, TASMANIA; School of Medicine, University of Tasmania\textsuperscript{2}, Hobart, TASMANIA.

Introduction. Renal insufficiency and advancing age decrease the glomerular filtration rate (GFR), which requires the dosage individualisation of many drugs to prevent adverse events. However, a significant percentage of patients with renal disease are administered inappropriately high doses of drugs (Markota et al, 2009). Lack of quantitative data in the available drug information sources and inconsistency in dosing information may augment the problem of dosing error.

Aims. To determine the consistency among five drug information sources regarding the dosage recommendations provided for drugs considered problematic in patients with renal impairment.

Methods. All five drug information sources viz. British National Formulary, American Hospital Formulary System Drug Information, Australian Medicines Handbook, Medical Information Management System, and Drug Prescribing in Renal Failure (published by American College of Physicians) were reviewed and information on recommendations for dosage adjustment in renal impairment was extracted and analysed for 61 drugs recommended as requiring caution (Veterans’ MATES, 2012).

Results. Recommendations tended to vary in the information sources. Only moderate agreement between the sources was observed (inter-rater reliability: Fleiss Kappa: 0.3; average pairwise percent agreement: 67%). Qualitative data were not well defined and there was lack of consistency in quantitative values. Drugs including glibenclamide, metformin and codeine were marked as contraindicated in one source but not mentioned in others. Also, drugs considered as not requiring dosage adjustment in one source (e.g. teriparatide, candesartan and bupropion) had explicit recommendations in other sources.

Discussion. There should be an evidence-based approach on drug dosage adjustment in order to bring uniformity to the recommendations. Regular updating of the content of the drug information sources is also important.

Veterans’ MATES (2012) \url{www.veteransmates.net.au/VeteransMATES_TherBrief.pdf}
Development and validation of software to calculate the drug burden index: a pilot study
Lisa M Kouladjian1,2, Danijela Gnjidic1,3, Sepehr Shakib4, Timothy F Chen3, Sarah N Hilmer1,2. Department of Clinical Pharmacology and Aged Care, Royal North Shore Hospital1, St Leonards NSW. Sydney Medical School, University of Sydney2, Sydney NSW. Department of Clinical Pharmacology, Royal Adelaide Hospital4, Adelaide SA

Introduction. The Drug Burden Index (DBI), a novel pharmacologic risk assessment tool that measures an individual’s total exposure to anticholinergic and sedative medicines, has been associated with impaired physical and cognitive function, frailty, falls and increased hospitalisation in older adults.

Aims. (1) To develop software which calculates and generates reports on DBI; (2) To use data from fictitious patients to test this DBI software for accuracy.

Methods. Based on previous work by Dr Sepehr Shakib, Microsoft Access 2010 was used to build and design The DBI Calculator©. Fictitious patient data was collected from the Scenarios of the Disease State Management Education Sections of the Australian Journal of Pharmacy (Aug 2010 to Aug 2012). The fictitious data was used to calculate DBI using the manual calculation and The DBI Calculator©. Cohen’s Kappa statistics were used to calculate the degree of concordance between manual and automated DBI scores.

Results. The software has been designed with a front and a back end; the front end allows the user to enter data, the back end allows for data management and analysis. The software design allows for ease of uploading onto a secured, de-identified, password-protected website. The user enters patient data and clicks “Calculate DBI” which immediately reports the DBI with the significance of the calculation for the patient. Preliminary results indicate good agreement between the software and manual calculation (Cohen’s Kappa 0.81) of the 16/25 fictitious patient scenarios used with DBI>0.

Discussion. We have developed a reliable calculator to report DBI in older patients taking multiple medications. Further studies will assess usability and application of The DBI Calculator© in clinical settings such as Home Medicine Reviews.


Identifying effective strategies to prevent drug-drug interactions in hospital: a user-centered approach
Olivia A Missiakos1,2, Melissa T Baysari2,3, Richard O Day1,2. UNSW Medicine1, Kensington, NSW; Dept of Clin Pharmacol, St Vincent’s Hosp2, Darlinghurst, NSW; Australian Institute of Health Innovation UNSW3, Kensington, NSW.

Introduction. Drug-drug interactions (DDIs) are an important, yet preventable cause of medication errors in hospitals. Research has shown that doctors and pharmacists are often unable to recognise potential DDIs. To prevent DDIs, numerous strategies, including alert systems, reference sources, and personalised prescriber feedback have been implemented worldwide. These strategies are rarely evaluated and are typically implemented without input from the individuals using those strategies.

Aims. To ascertain the most appropriate mechanism to prevent DDIs in a hospital, as viewed by users.

Methods. Eight drug safety experts and 18 doctors took part in semi-structured interviews. Participants were asked about their confidence in identifying DDIs, their opinion on currently used strategies, as well as their views on possible future strategies to prevent DDIs. Interviews continued until saturation of themes was achieved. Transcripts were analysed and coded to identify key themes and compare groups.

Results. Reference sources and ward-pharmacists were consistently consulted as current DDI prevention strategies, however users also identified multiple limitations with these strategies. No doctors reported being completely confident in identifying dangerous DDIs and junior doctors were less confident in their ability than senior doctors. Most doctors thought that alert systems would be an effective strategy to implement. This was because alerts would highlight potential problems in situations where individuals were too busy, or not cognisant of the possibility of DDIs.

Discussion. The lack of confidence by doctors regarding DDIs suggests that a strategy which doesn’t rely on individuals seeking the information out for themselves would be most appropriate at this site. While the literature has identified numerous problems with the implementation of DDI alerts, this study showed that users were receptive to the idea. By involving users in DDI strategy design we expect greater policy adherence and satisfaction.
**Impact of pharmacist-led dosing of vancomycin and aminoglycosides in hospitalised patients**

Daniel O’Brien,1,2 Erica Tong,1 Shin Choo,1,3 Carmella Corallo,1 Kelly Cairns,1 Susan Poole,1,2 Allen Cheng,3,4 Michael Dooley,1,2 Pharmacy Dept, Alfred Health1, Melbourne, Vic; Faculty Pharmacy & Pharmaceutical Sciences, Monash Univ2, Melbourne, Vic; Dept Epidem & Prevent Med, Monash University3, Melbourne, Vic; Infectious Disease Unit, Alfred Health4, Melbourne, Vic. (*introduced by Carl MJ Kirkpatrick, Monash Univ, Melbourne, Vic)

Introduction. Aminoglycosides and vancomycin are commonly used antibiotics and display narrow therapeutic indices and inter-patient-variable pharmacokinetics. Therefore, hospital practice is to perform dose individualisation and therapeutic drug monitoring (TDM) with the aim of achieving and maintaining therapeutic concentrations. TDM and dosing is primarily the responsibility of the prescriber, with variable input from clinical pharmacists.

Aim. To determine the impact of pharmacist-led dosing for vancomycin and aminoglycosides across a multisite health service.

Methods. A pre- and post-intervention cohort study was conducted (150 patients pre and post). The intervention involves clinical pharmacists ordering drug-levels and independently adjusting doses once therapy has been initiated by the treating medical team. Clinical pharmacists were trained, assessed and accredited to provide the service 7-days per week. Endpoints included the time to first therapeutic level; proportion of patients reaching therapeutic level and the appropriateness of TDM (a composite measure of correct timing of levels and subsequent dosage adjustments).

Results. 300 patients were included (100 vancomycin and 50 aminoglycoside courses pre and post-intervention). Patient characteristics, duration of therapy, indication and number of levels were similar across the pre- and post cohorts. For patients receiving vancomycin the time to first therapeutic level was significantly less for the pharmacist-led dosing group (1.9 vs 2.6 days, p=0.008); the proportion of patients ever reaching therapeutic levels was similar (77% for pharmacist-led vs 72% for physician-led, p=0.41); the proportion of dosing adjustments fully compliant with specific criteria in the guideline was significantly greater in the pharmacist-led dosing group (60% vs 50%, p=0.004).

Discussion. The pharmacist-led service has resulted in a significant improvement in the time to reach therapeutic level and correct dose adjustments. This is now an established service, provided as a routine component of clinical pharmacists’ practice and is a model for further expansion.
Development of a population PKPD model to describe the effect of paracetamol on the International Normalised Ratio (INR).

Katie H Owens1, Natalie J Medlicott1, Ian M Whyte2, Nicholas A Buckley3, David M Reith4. School of Pharmacy, Univ of Otago1, Dunedin, NZ; School of Medicine and Public Health, Univ of Newcastle, Newcastle, NSW2; Faculty of Medicine, Univ of New South Wales, Sydney, NSW3; Dunedin School of Medicine, Univ of Otago, Dunedin, NZ4.

Introduction. Paracetamol is one the most common substances taken in overdose. (Reith et al, 2009) Paracetamol may increase International Normalised Ratio (INR) in paracetamol poisoning without hepatic injury by reducing functional factor VII. (Whyte et al, 2000)

Aims. The aim of this study was to develop a population model to describe the PKPD of paracetamol and its effect on INR.

Methods. A total of 167 patients were included in the dataset (31 paracetamol overdose patients, 9 control overdose patients, 20 cross-over clinical trial patients, 107 retrospective paracetamol overdose patients); 63 were men, the median age (range) was 22 years (13–71). A structural population PKPD model was developed in Phoenix® NLME™. 167 patients contributed a total of 907 paracetamol plasma and INR observations.

Results. The pharmacokinetics of paracetamol were best described by a 1-compartment model with first order input and linear disposition. A modified baseline Emax model described the effect of paracetamol on INR by inhibition of the activation of vitamin K-dependent coagulation factors. The population mean estimates ± SE (CV%) of the pharmacokinetic parameters volume of distribution, clearance, absorption rate constant (Ka), and lag time (tlag) were 10.6 ± 1.8 (17) L, 2.3 ± 0.3 (13) L·h⁻¹, 3.1 ± 5.3 (172) h⁻¹, and 0.2 ± 0.1 (36) h, respectively. The population estimates ± SE (CV%) of the pharmacodynamic parameters Emax and EC50 were 0.4 ± 0.1 (30) (increase in INR) and 512 ± 256 (50) μM, respectively. An additive residual error model was used. Covariates investigated in the final model included age, sex, and treatment with N-acetylcysteine (NAC).

Discussion. Preliminary findings demonstrated that the model adequately estimated population PKPD parameters for paracetamol and provided the basis for covariate analyses.


Assessment of frailty and prescribing criteria in older people: A systematic review

Arjun Poudel1, Lisa Nissen2, Ruth E Hubbard3, Charles Mitchell4. School of Pharmacy, The University of Queensland1,2, Brisbane, QUEENSLAND; School of Medicine, The University of Queensland3, Brisbane, QUEENSLAND; Centre for Safe and Effective Prescribing, The University of Queensland4, Brisbane, QUEENSLAND

Introduction: Rational prescribing (RP) in frail older people is complex and difficult. In this group, there is limited evidence on effectiveness of medication, drug pharmacokinetics and pharmacodynamics differ from younger people and multiple co-morbidities with higher risk of adverse drug events are more likely (Couteur et al, 2004). Various criteria have been developed to measure inappropriate prescribing but their applicability to frail older population is uncertain (Gnjidic & Hilmer, 2010).

Aims: The primary aim of this systematic review was to identify studies describing the use of frailty measures for evaluating inappropriate prescribing in those aged 65 and older. The secondary goal was to address the missing parameters in the prescribing tools to increase their utility for frail individuals.

Methods: A search was conducted in PubMed and EMBASE (1990- 2011). Original studies written in English that utilized frailty assessment and criteria to evaluate inappropriate prescribing in frail individuals were included. Excluded are: studies of specific drugs or groups of drugs and of particular disease conditions.

Results: Ten of 573 studies met the inclusion criteria. All papers measured certain parameters of frailty, such as performance based tests, measures of co-morbidity, etc. Six studies used the Beers criteria to explicitly identify inappropriateness while Medication Appropriateness Index (MAI); an implicit criteria was used by two studies and combination of both in other two studies.

Discussion: Although some parameters of frailty measurement have been used, there appears a need of more user-friendly and detailed criteria for assessing frailty in older individuals. Prescribing tools should address both medication and patient related factors such as life expectancy and functional status to minimize inappropriate prescribing in frail individuals.

Gnjidic D, Hilmer SN, (2010), Aging Health, 6(6):705-16
**Association between age, lean body weight, frailty and inducible SIRT1 expression response to sera: the CHAMP study**

Shajjia Razi¹³, Victoria Cogger¹², David G Le Couteur¹², Vasi Naganathan¹², Vicky Benson³. Anzac Research Institute¹, Centre for Education and Research on Ageing², University of Sydney³, Concord, NSW.

Introduction. Tissue SIRT1 expression and activity increase with caloric restriction and often decrease in old age. Sera from either middle age rats or humans subjected to caloric restriction increase SIRT1 expression in cultured cells. This suggests that there are circulating factors that influence SIRT1 expression and potentially correlate with ageing and age related health.

Aims. To determine the effect of frailty in older people on such circulating factors.

Methods. We measured SIRT1 expression in cells induced by culture in presence of sera from nested group of subjects from CHAMP, a study of community dwelling males greater than 70 years of age.

Results. Inducible SIRT1 expression was not different between frail and robust subjects. However subjects with expression values in the lowest quartile of values were likely to be frail and had higher lean body weight.

Discussion. This suggest that low expression of SIRT1 induced by sera is paradoxically associated with some markers of better health in older men.
An Evaluation of Switches in Therapeutic Equivalents as Triggers for Adverse Event Monitoring
Andrew Tomlin 1, David Reith 2, Susan Dovey 2, Murray Tilyard 2. Best Practice Advocacy Centre 1, Dunedin, NEW ZEALAND, Department of Women’s and Children’s Health, and Department of General Practice and Rural Health, Dunedin School of Medicine, University of Otago 2, Dunedin, NEW ZEALAND

Introduction: General Practice electronic clinical records contain data that could be used to identify adverse events. Trigger tools could be developed to help identify when adverse events have occurred.

Objective: To examine changes in a therapeutic equivalent (antidepressant) in relation to reports of adverse drug events (ADEs) and symptoms indicative of ADEs within general practice electronic records.

Methods: Electronic clinical records for a cohort of 338,931 patients consulting from 2002-2007 were extracted from the patient management systems of 30 primary care clinics in New Zealand. A structured chronological analysis of prescriptions, consultation notes and adverse events relating to patients prescribed the SSRI citalopram was undertaken included investigating reasons for switching treatment to another SSRI (fluoxetine or paroxetine) as a method for detecting evidence of ADEs.

Results: During the study period 173,478 patients received 4,811,561 prescriptions. Citalopram was prescribed for 5,612 patients and 610 adverse reactions to citalopram were identified in the consultation and medical warning records of 397 (7.1%) patients. A total of 713 (12.7%) patients changed treatment from citalopram to another SSRI.

Reasons for switching were identified for 164 patients: ADE for 129 (78.7%), lack of effect for 29 (17.7%) and patient preference for six (3.7%). The most common ADEs preceding the switch were anxiety, nausea and headaches.

Conclusions: A switching of therapeutic equivalent can be used as a trigger for reports of ADEs.

Measuring drug concentrations, analytical methods and issues
Andrew Rowland1, David J Elliot2, Matthew Doogue1,3.
Dept of Clin Pharm, Flinders University1, Bedford Park, SA. Chem Path Dir, SA Pathology2, Bedford Park, SA. Dept of Clin Pharm, Flinders Medical Centre3, Bedford Park, SA.

Introduction. The primary goal of Therapeutic Drug Monitoring (TDM) is to improve patient outcomes. In order to support this goal, the analytical approach underpinning TDM must be robust and reproducible, requiring validation to a standard suitable for regulatory approval.

Discussion. TDM is most commonly used for small drug molecules and their metabolites measured in plasma/serum or whole blood. However many other matrices are used and increasingly large molecule “biological” drugs are being used in clinical practice. There are a number of analytical techniques that can be used to measure drug concentrations and these will be the focus of this presentation. These techniques typically involve first separating the compound of interest form its biological matrix (plasma, whole blood or urine); this may be achieved through high (or ultra) performance liquid (or gas) chromatography (HPLC / UPLC / GC). Once the compound of interest has been isolated, it is then quantified either by measurement of ultra-violet absorbance, fluorescence or by mass spectrometry. When developing and validating an analytical method for use in TDM there are several key factors that must be consider. These are: accuracy (bias and imprecision), specificity, limit of detection, limit of quantitation/identification, linear dynamic range, reproducibility, repeatability, robustness and timeliness. Selection of an analytical approach for undertaking TDM must therefore be based on fitness for purpose and the capacity of the technique to address these factors in a manner appropriate to the drug being measured and does not necessarily require the use of the most sophisticated or modern technology.
New Zealand Formulary - a case study of national formulary development
Bryan H Simpson1, Duncan S T Enright2, Catherine E Herd1, Amber F Lauder1, Murray W Tilyard1, David J Woods1. New Zealand Formulary1, Dunedin, New Zealand; British National Formulary, Pharmaceutical Press2, London, United Kingdom.

Introduction. The New Zealand Formulary (NZF) was developed to build on the New Zealand Universal List of Medicines through the addition of clinical information about medicines use. The structure and content of the NZF is based on that of the British National Formulary (BNF) but is adapted for New Zealand practice. It contains key information on the selection, prescribing, dispensing, and administration of medicines available as an online resource.

Aim. To adapt the BNF to provide New Zealand (NZ) healthcare professionals with information about the selection, prescribing, dispensing, and administration of medicines by July 2012.

Methods. The initial release of the NZF was adapted from the latest version of the BNF focusing on relevance to NZ practice. The BNF therapeutic notes were reviewed by medical specialist advisors and clinical pharmacists before they were signed-off by an editorial advisory board. The BNF monographs were compared to NZ approved Medicine Datasheets (NZAMD) and tailored to reflect NZ approved indications and doses. Other fields of the monographs were also reviewed and additional detail and practical advice was added where appropriate including inclusion of Stockley’s interaction alerts and Australian eTG breast-feeding categories.

Results. The NZF was successfully developed within the specified contract timeframe and budget. It was also being utilised by the health sector with 57,809 visitors in the first 3 months. Also, in the first 3 months the average number of daily users has consistently increased to 713. Clinical feedback is divided into domains that are evaluated for future enhancements.

Discussion. The BNF can be successfully adapted in a timely and cost effective manner to the NZ context.


Evaluation of formulary applications in Australian paediatric hospitals
Yashwant K Sinha1,2, Jonathan C Craig1,3, Peter Barclay4, Jo-anne E Brien2,5,6. Centre for Kidney Res1, The Children’s Hospital at Westmead, Westmead, NSW, Faculty of Pharmacy2, Univ of Sydney, Camperdown, NSW, School of Public Health3, Univ of Sydney, Camperdown, NSW, Pharmacy Dept4, The Children’s Hospital at Westmead, Westmead, NSW, Therapeutics Centre5, St Vincents Hospital, Darlinghurst, NSW, Faculty of Medicine6, Univ of NSW, Kensington, NSW

Introduction. Evaluation and approval of drugs for use in hospitals is a primary objective of hospital drug and therapeutics committees. Despite international legislative and regulatory changes, the evidence base informing care for children remains poor, compared with adults. There is limited information in the published literature regarding the evaluation of drug approval processes in paediatric hospitals.

Aims. 1. To evaluate the quality of applications for addition to the formulary in Australian paediatric hospitals. 2. To evaluate the data supporting paediatric formulary applications including committee decisions. 3. To identify gaps in research relating to use of drugs in children and policy implications.

Methods. Multicentre descriptive study involving review of committee documents and drug submissions for all Australian paediatric hospital Drug and Therapeutics Committees over an 18 month period. Main outcome measures: application format, type of supporting literature, committee decision, declaration of conflict of interest.

Results. All eight paediatric hospitals agreed to take part. The total number of formulary applications was 121. Approval rates varied from 58-100% for each hospital. To date, we have analysed 88 (73%) applications and found that most applications (68%) were formally submitted using a standardised template by medical staff (65%). 39% of applications underwent independent review by a statewide medicines advisory committee or hospital pharmacist. A conflict of interest was declared for 9 (10%) applications. Quality of supporting data varied with many applicants including the product information, therapeutic guidelines and review papers as the predominant literature.

Discussion. These data confirm previously reported high approval rates for paediatric formulary submissions (Sinha YK et al 2010). Our preliminary findings suggest there is limited high quality evidence informing hospital-based drug approvals. This study provides a new national dataset relating to therapeutic decision making in tertiary paediatric hospitals.

Diagnostic errors in older patients: A systematic review of the incidence and causes in thirteen prevalent conditions.
Thomas R Skinner\(^1\), Ian A Scott\(^1,2\), Jennifer H Martin\(^1,2\). University of Queensland, Brisbane, QLD\(^1\). Princess Alexandra Hospital, Brisbane, QLD\(^2\).

Background: Misdiagnosis, either over or under-diagnosis, exposes older patients to increased risk of inappropriate or omitted investigations and treatments, psychological distress and financial burden.

Objectives: To evaluate the frequency and causal factors of diagnostic errors relating to thirteen conditions prevalent in older patients.

Data sources and study selection: Cohort studies, cross-sectional studies or systematic reviews of such studies published in Medline between January 2001 and July 2011 were searched using key search terms of “diagnostic error” “misdiagnosis” “accuracy” “validity” or “diagnosis” and terms for each disease.

Data synthesis: Of 1260 retrieved articles, 29 were selected for inclusion. Rates of over-diagnosis and under-diagnosis were as high as 69% and 71% respectively with some conditions both under- and over-diagnosed. Over-diagnosis rates of more than 10% were seen in chronic obstructive pulmonary disease (18% - 35%), stroke and transient ischaemic attack (11% - 71%), major depression (22% - 69%), Parkinson’s disease (1% - 27%), heart failure (1% - 64%) and epilepsy (16%). Under-diagnosis rates of more than 10% were seen in ischemic heart disease (2% - 34%), major depression (12% - 53%), Parkinson’s disease (9% - 47%), heart failure (4% - 71%) and epilepsy (16% - 27%).

Conclusion: Diagnostic errors involving older patients are common and comprise both over-diagnosis and under-diagnosis. Explanations for over-diagnosis include the subjective interpretation of clinical diagnostic criteria and use of criteria which have not been validated in older patients. Under-diagnosis was associated with long pre-clinical phases of disease or lack of sensitive diagnostic criteria. Factors that predispose to misdiagnosis in older patients must be given emphasis in training programs and clinical practice guidelines.

An evaluation of a change in dosing regimen of gentamicin in neonates
Louise A. Thomas\(^1\), Natalie J. Medlicott\(^1\), Roland Broadbent\(^2\), David M. Reith\(^2\). \(^1\)New Zealand National School of Pharmacy, University of Otago, Dunedin, New Zealand; \(^2\)Dunedin School of Medicine, University of Otago, Dunedin, New Zealand.

Introduction. It has previously been demonstrated that clinically significant variability and delay in the administration of gentamicin occurs in neonates and is related to the method of administration.\(^1\)

Aim. To compare the effects of two dosing methods on the delivery of gentamicin to neonates.

Methods. Two dosing methods were compared; bolus and a 30 minute infusion. Neonates admitted to Dunedin Neonatal Intensive Care Unit (NICU), in 2008 and 2010 that had a gentamicin dose in the first three days of life and a one hour peak and 23 hour trough level, were included in the study. A pharmacokinetic (PK) analysis was performed with Phoenix NLME using a zero order input and first order elimination. Duration (Tinf1=2010, Tinf2=2008) of infusion was parameterised separately for each dosing method. The model was optimised using a covariate approach.

Results. There were 73 patients in 2010, 31 females and 42 males; and 97 patients in 2008, 36 females and 61 males. The median and range values were: 2010; gestational age (GA) 37.714weeks (24.286-41.857) and weight (W) 3.165kg (0.51-5.65); 2008; GA 34.643weeks (23.429-42) and W 2.413kg (0.49-5.105). The parameter estimates were: \(V = 1.205 * (W/mean(W))^0.835 * \exp(nV))\); \(Cl = 0.097 * (W/mean(W))^0.9015 * (GA/mean(GA))^1.233 * \exp(nCl))\); Tinf1 = 1.05; Tinf2 = 0.901. The magnitude of inter-patient variability, expressed as CV% were <20%. The magnitudes of residual error in the dosing methods were, 0.414 for Tinf1 and 0.469 for Tinf2.

Discussion. There was no significant difference in the duration of the infusion between the two years. This suggests further PK analysis is required on the input of gentamicin in neonates.

\(^1\)Sherwin C. et al. (2009) JPP 61:465-461
**Monitoring the Anti-Proteus activity of Colloidal Metallic Silver (CMS)**

Michael W Whitehouse¹, Mihails A Baharevs², Michael W Whitehouse¹, Mihails A Baharevs². Biomolecular Sciences¹ and Environmental Futures², Griffith University (Nathan campus), Brisbane, Qld.

Introduction. Colloidal silver has been used as an antibiotic for over 100 years (Cock et al 2012). Patients with rheumatoid arthritis may produce antibodies to both *Proteus* bacteria and to proteins containing citrullinyl residues. *Proteus* are enterobacteria also found in the upper urinary tract (notably in females), that can transform protein-arginyl residues to antigenic protein-citrullinyl residues.

Aims: To investigate antibiotic efficacy of CMS against an arthritigenic bacterium.

Methods. Anti-arthritic activity was evaluated after orally administering colloidal metallic silver (CMS) to female Wistar rats developing chronic polyarthritis after tailbase injection of either (i) a complete Freund’s adjuvant or (ii) collagen type-II with an incomplete Freund’s adjuvant or (iii) pristane. Anti-proteus activity was determined by a disc diffusion assay growing *P. vulgaris* and *P. mirabilis* on agar plates in the presence of various CMS preparations.

Results. A. CMS preparations made electrolytically and administered orally (alternate days for two weeks) were powerful anti-arthritic agents in rats (ED₉₀ approx. 85 μg/kg total silver). Monovalent silver products (acetate, nitrate, oxide) were ineffective at twice this dose.

B. CMS preparations were also more potent than silver salts in suppressing growth of *Proteus sp. in vitro*. Against *P. mirabilis*, minimal inhibitory concentrations (MIC) of total silver were greater than 22μg/ml for chemically prepared CMS and less than 30μg/ml for electrolytically prepared CMS: the difference being due to the smaller size of nanoparticles and different Zeta potentials in electrolytic preparations, compared with chemical preparations, of CMS.

Discussion. Pro-arthritic gut micro-organisms may be susceptible to ‘old’ antibiotics taken orally such as colloidal silver, as well as to accepted slow-acting anti-rheumatic drugs (DMARDs) originally developed as antibiotics eg minocycline, salazopyrine. More rigorous Quality Controls must be developed for the preparation of CMS – as well as antimicrobial efficacies and general safety.

**Evaluating posaconazole use in a patient population.**

William Wu¹², Shaun S Kumar¹², Brian J Egan¹, Kenneth M Williams¹², Deborah J.E Marriott¹³, Richard O Day¹². Dept of Clin Pharmacol, St. Vincent's Hosp¹, Darlinghurst, NSW; School of Medical Sciences, Univ of New South Wales², Kensington, NSW; Dept of Clin Micro & Infectious Diseases, St. Vincent’s Hosp³, Darlinghurst, NSW.

Introduction. Posaconazole is approved for prophylactic use against invasive fungal infections (IFIs) and treatment of refractory infections. It exhibits highly erratic absorption partly due to its high lipophilicity. Oral availability is reduced by proton pump inhibitors (PPIs) and pro-gastric motility agents. Absorption is increased by concomitant food intake and by dividing the daily dosage. Therapeutic dosage monitoring (TDM) is recommended to maintain plasma concentrations above a putative target of 700 ng/ml.

Aims. (1) To determine if adequate plasma concentrations are achieved in patients at risk (prophylaxis) or being treated for fungal infection and, (2) identify any drug-drug interactions or clinical factors which alter concentrations.

Methods. An audit of patients prescribed posaconazole at the study site was undertaken for the period June 2007 to June 2012. TDM data and patient records were reviewed. Concentrations were excluded if records indicated missed dose prior to sampling. Proven or probable IFIs were identified in the prophylactic cohort. Data are presented as median and 95% CI.

Results. Seventy-eight patients were prescribed posaconazole. From 68 patients, 511 plasma concentrations were available. Median concentrations were <700 ng/ml in 48 patients, 6 of these patients continued with posaconazole despite low concentrations and ongoing neutropenia. Low concentrations were observed with concomitant PPI usage (369 [209-542] ng/ml vs. 620 [346-1183] ng/ml, p<0.0001, with and without PPI, respectively; n= 34 patients). In-patient concentrations were significantly lower than out-patient (470.5 [298-731] ng/ml vs. 1021 [419-1587] ng/ml, p<0.0001). Six of 62 patients prescribed posaconazole for prophylaxis failed therapy, the median of their concentrations were similar, though lower, than for patients who did not have a fungal breakthrough (446 [265-808] ng/ml vs. 531 [327-1042] ng/ml, p=0.18).

Discussion. Sub-therapeutic levels of posaconazole were common. Concomitant PPI usage and in-patient status were associated with sub-therapeutic concentrations. Guidelines involving TDM are needed to improve posaconazole’s use.
**Electronic prescribing in St Vincent’s hospital and the National Inpatient Medication Chart audit**

Brian J Egan1, Mark O’Reilly1, Kate Richardson2, Jane Ludington2, Sarah McLain2, Kelly Tank3, Nicola Jackson2, Maureen Heywood2, Ric O Day1,1. Department of Clinical Pharmacology and Toxicology, St Vincent’s Hospital, Sydney, Australia 2. Pharmacy Department, St Vincent’s Hospital, Sydney, Australia 3. Department of Clinical Information Systems, St Vincent’s Hospital.

Introduction. The National Inpatient Medication Chart (NIMC) Audit System assists Australian hospitals auditing their use of the NIMC. The nationally coordinated NIMC audit occurs every two years. In 2005, St Vincent’s Hospital (SVH) introduced an electronic medication management system (eMMS) and now every ward of the hospital is using the eMMS.

Aims. To compare St Vincent’s Hospital, Sydney NIMC Audit data with the national data and identify areas of good medication management practice and how these relate to an electronic Medication Management System.

Methods: On August 28th 2012 we audited 141 patient medication charts in SVH using the NIMC audit form. We used the Australian Commission on Safety and Quality in Health Care NIMC audit website to generate Excel reports of the national and local results of the NIMC audit.

Results. 9689 patient medication charts in 312 hospitals were audited. In St Vincent’s Hospital 121 electronic charts from inpatient wards and 20 paper charts from the emergency department were audited. Figure 1 identifies a number of differences between medication chart documentation in SVH and the national data. 50% of patients in SVH were prescribed VTE Prophylaxis compared with 19% nationally. Some parameters are not applicable to the electronic medication management system and were not recorded.

Discussion. A forcing function in eMMS of Adverse Drug reactions has resulted in better documentation in St Vincent’s Hospital and this could be applied to other parameters. Pharmacy Annotation was markedly better on electronic charts. Documentation of weight and indication could be improved at SVH.


**Kavalactones: Novel positive modulators of α2β1γ2L GABA\(_{\text{A}}\) receptors**

Emilie Christensen1, Nathan Absalom1, Iqbal Ramzan1, Mary Chebib1. Faculty of Pharmacy, The University of Sydney 1, Sydney, NSW.

Introduction. Kava (Piper methysticum) extract has gained popularity as an anxiolytic intoxicating beverage due to its anxiolytic effects. Previous studies indicate that kavalactones affect a variety of molecular targets including the inhibitory ionotropic GABA\(_{\text{A}}\) receptors. (Rowe A et al, 2011)

Aims. Characterizing kavalactones on GABA\(_{\text{A}}\) receptors.

Methods. The effect of kavalactones (kavain, yangonin, methysticin and dihydromethysticin) was evaluated on recombinant α1β1γ2L (x=1-3) GABA\(_{\text{A}}\) receptors expressed in Xenopus oocytes using two electrode voltage clamp.

Results. The kavalactones (100 μM) had no effect alone or as modulators of GABA on α1β1 and α1β1γ2L but enhanced the action of GABA (EC\(_{50}\)) at α1β1γ2L by 65-80%. The EC\(_{50}\) of kavain was not significant differently (P>0.05, t-test) between α1β1γ2L (EC\(_{50}(α1β1γ2L) = 44.23 [95%CI 22.24-87.96, n=7]μM; EC\(_{50}(α1β1γ2L) = 63.23 [95%CI 49.05-81.51, n=3]μM) and α1β1γ2L (EC\(_{50}(α1β1γ2L) = 29.67 [95%CI 11.17-78.78, n=8]μM; EC\(_{50}(α1β1γ2L) = 60.11 [95%CI 36.62-98.66, n=8]μM). Flumazenil (0.1-10 μM) did not block the potentiation of GABA by kavain on α1β1γ2L. The efficacy (E\(_{\text{MAX}}\)) of kavain/yangonin was reduced 2-5 fold at α1β1γ2L by 65-80%. The EC\(_{50}\) of kavain/yangonin was not significant differently from the wild-type (α1β1γ2L) = 44.23 [95%CI 22.24-87.96, n=7]μM; EC\(_{50}(α1β1γ2L) = 63.23 [95%CI 49.05-81.51, n=3]μM) and α1β1γ2L (EC\(_{50}(α1β1γ2L) = 29.67 [95%CI 11.17-78.78, n=8]μM; EC\(_{50}(α1β1γ2L) = 60.11 [95%CI 36.62-98.66, n=8]μM). Flumazenil (0.1-10 μM) did not block the potentiation of GABA by kavain on α1β1γ2L. The efficacy (E\(_{\text{MAX}}\)) of kavain/yangonin was reduced 2-5 fold at α1β1γ2L by 65-80%.

Discussion. Kavalactones are not binding to the benzodiazepine site but act as positive modulators of GABA\(_{\text{A}}\) receptors at both α1β1γ2L and α1M236W may be involved in either receptor gating and/or binding of kavalactones.

**Rat model of varicella zoster virus (VZV) induced neuropathic pain**

Vaskar Das¹ ², Ai-Leen Lam¹ ² and Maree T Smith¹ ². Centre for Integrated Preclinical Drug Development¹ and School of Pharmacy, The University of Queensland², Brisbane, QLD.

Introduction. Pain that persists for greater than 3 months after the shingles rash has healed is known as postherpetic neuralgia (PHN), a condition notoriously difficult to treat. Hence, there is a large unmet medical need for new treatments to alleviate PHN.

Aim. To establish a rat model of VZV-induced neuropathic pain for assessing the analgesic efficacy of novel molecules for neuropathic pain relief.

Methods. The Ellen strain of VZV was propagated in vitro in cultured MRC-5 cells to ~80% confluence. VZV infection of MRC-5 cells was confirmed by RT-PCR and Western blot using an antibody against the VZV gE protein. Adult male Wistar rats were randomized to one of three groups (n=4-6 per group) that received unilateral intraplantar injections (50 µL) of: (i) Phosphate buffered saline (pH7.4, 1mM, control group), (ii) MRC-5 cells (7x10⁶ cells/ml; sham group) or (iii) VZV-infected MRC-5 cells containing 10⁷ plaque forming units. Von Frey filaments were used to define the time course for the development of mechanical allostynia in the hindpaws and to assess the analgesic effects of single bolus subcutaneous doses of gabapentin at 10, 30 & 60mg/kg. A dose-response curve was generated and the ED₅₀ was estimated using nonlinear regression (GraphPad Prism™ v.5.03).

Results. VZV infection of MRC-5 cells was confirmed by RT-PCR and Western blot. Bilateral mechanical allodynia was fully developed in the hindpaws of VZV-infected animals (paw withdrawal thresholds ≤ 6g) by day 7 and maintained until at least day 35. Gabapentin produced dose-dependent relief of hindpaw hypersensitivity and the mean ED₅₀ was 25.0mg/kg.

Discussion. A VZV-induced rat model of neuropathic pain has been established.

---

**Balb/c, C57BL/6j and CBA mice: characterisation of a new population of MDMA (ecstasy) users**

Jake J Gordon¹, Mark R Hutchinson², Rodney J Irvine¹, Abdallah Salem¹. Disc of Pharmacol¹, School of Medical Sciences, Univ of Adelaide, Adelaide, SA; Disc of Physiol², School of Medical Sciences, Univ of Adelaide, Adelaide, SA

Introduction. MDMA-induced hyperthermia is the major feature in acute toxicity cases and is known to potentiate the neurotoxic effects of MDMA. Although there is a well documented role for 5-HT and dopamine (Mechan et al, 2002), microglial activation has recently been implicated in potentiating MDMA-induced hyperthermia (Orio et al, 2004). We propose to exploit inherent differences in mouse strains to further explore the involvement of microglia activation in MDMA-induced hyperthermia.

Aims. To develop a novel model in three mouse strains to investigate the mechanisms behind MDMA-induced hyperthermia.

Methods. Male Balb/c, C57BL/6j and CBA mice were administered MDMA (20 and 40 mg/kg, i.p) and body temperature and locomotor activity were monitored for 4 h at an ambient temperature of 22-23.5 °C.

Results. MDMA administered in Balb/c mice was shown to decrease body temperature significantly compared to saline (n=7-8, P<0.01), with 20 mg/kg MDMA seen to decrease body temperature more rapidly than 40 mg/kg MDMA. When given in C57BL/6j mice, MDMA increased body temperature significantly compared to saline (n=8-10, P<0.001), however there was no significant difference between 20 and 40 mg/kg MDMA. MDMA given in CBA mice produced a significant dose dependent increase in core body temperature (n=4-10, P<0.001). MDMA was seen to significantly increase locomotor activity in all strains at a dose of 40 mg/kg when compared to saline controls (n=4-10, P<0.05), however at a dose of 20 mg/kg, only C57BL/6j and CBA mice showed significantly increased locomotor activity (n=4-10, P<0.05).

Discussion. These results clearly display heterogeneity of response to MDMA in three mouse strains, with respect to both body temperature and locomotor activity. The results validate the model by providing a foundation on which to investigate the underlying neurochemical and inflammatory causes of MDMA-induced hyperthermia.


**Stress-reducing effect of GABA-enriched tea in humans: assessment of stress using heart rate variability**

Sin Yoo Kam, Tina Hinton, Slade Matthews, Graham Johnston. School of Medical Sciences (Pharmacology), Univ of Sydney, NSW.

Introduction. GABA-enriched dietary supplements are purported to address chronic stress-induced autonomic imbalance as a risk factor for cardiovascular disease. However, there is insufficient evidence to support the effectiveness of exogenous GABA intake.

Aims. To investigate the acute effects of GABA-enriched tea in reducing stress induced by a mental arithmetic stressor in individuals, as detected by heart rate variability (HRV).

Methods. Participants were randomly allocated to consume GABA-enriched oolong tea (n=17), regular oolong tea (n=17) or water (n=17). HRV was assessed by electrocardiogram (ECG) conducted at baseline, during a 2-min mental arithmetic stressor task and after the stressor.

Results. The mental arithmetic stressor significantly decreased high frequency (HF) component of HRV (2.87±0.13 to 2.66±0.11; p<0.05) in the water group, while no significant differences were detected in either of the tea groups. Administration of GABA-enriched oolong tea led to a significant increase in low frequency (LF) component from 2.68±0.047 to 2.95±0.08 (p<0.05) during stress. While recovery from the stressor was observed in all groups, GABA-enriched oolong tea and regular oolong tea groups both exhibited increased average RR interval compared to baseline, from 863.4±24.1 to 918.0±23.4 (p<0.01) and 870.7±26.0 to 952.7±28.9 (p<0.001), respectively.

Discussion. Decreased HF in the water group during stress indicated parasympathetic nervous system (PSNS) withdrawal and sympathetic dominance in response to stress. No significant effect found in the tea groups suggested tea consumption induced stress-reducing effects, regardless of which tea was consumed. These results were consistent with the increased post-stressor average RR interval in the tea groups, indicating increased PSNS activity in reducing stress. Increased LF in the GABA-enriched oolong tea group also reflected activation of PSNS, which dominates when measured in the supine position. These data provide evidence for stress-reducing potential of teas and greater effectiveness was seen with GABA-enriched tea.

**Medication overuse headache is a manifestation of opioid induced hyperalgesia: A neuroimmune hypothesis and novel approach to treatment.**

Jacinta Johnson¹, Mark Hutchinson², Desmond Williams³ & Paul Rolan¹,⁴. Discipline of Pharmacol, Univ of Adelaide¹, Adelaide, SA. Discipline of Physiol, Univ of Adelaide², Adelaide, SA. School of Pharm and Med Sci, Univ of South Australia³, Adelaide, SA. Pain and Anaesthesia Research Clinic, Royal Adelaide Hosp⁴, Adelaide, SA.

Introduction. Patients with chronic headache who consume large amounts of analgesics are often encountered in clinical practice. Excessive intake of analgesics is now considered to be a cause, rather than simply a consequence of frequent headaches, and the diagnosis “medication overuse headache” has been formulated. Despite the prevalence and clinical impact of medication overuse headache the pathophysiology behind this disorder remains unclear and current treatment options are sub-optimal.

Aim. To explore a potential role for glial activation in the pathophysiology of medication overuse headache.

Methods. Preclinical and clinical data from the literature were reviewed.

Results. Although most acute headache treatments have been alleged to cause medication overuse headache, here we conclude opioids are the drug class most strongly associated with worsening headache. Recent evidence indicates chronic opioid administration may exacerbate pain in the long-term by non-specifically activating Toll-Like Receptor-4 on glial cells, resulting in a pro-inflammatory state that manifests clinically as hyperalgesia (Hutchinson et al, 2011).

Discussion. We hypothesise that medication overuse headache is a specific form of opioid-induced hyperalgesia, which derives from a cumulative interaction between central sensitisation, due to repeated activation of nociceptive pathways by recurrent headaches, and pain facilitation due to opioid-induced glial activation. Treatment strategies directed at inhibiting glial activation may be of benefit in the management of medication overuse headache. Potential treatment options could include agents such as ibudilast, minocycline and (+)-naltrexone.

Hutchinson MR et al. (2011) Pharmacol Rev 63(3):772-810
Cannabinoid receptor interacting protein (CRIP\textsubscript{1a}) modulates CB\textsubscript{1} receptor mediated GIRK channel activation in AtT-20 cells
Nilushi S Karunaratne\textsuperscript{1}, Paul J White\textsuperscript{1}, Mark Connor\textsuperscript{2}, Daniel T Malone\textsuperscript{1}. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences\textsuperscript{1}, Melbourne, VIC; Australian School of Advanced Medicine, Macquarie Univ\textsuperscript{2}, Sydney, NSW.

Introduction. Endocannabinoids have unique analgesic and anxiolytic properties, largely mediated by activation of the cannabinoid CB\textsubscript{1} receptor in the central nervous system. Cannabinoid receptor interacting protein (CRIP\textsubscript{1a}) binds to and interacts with the C-terminal tail of the CB\textsubscript{1} receptor (aa 418-472) and has been shown to suppress the tonic inhibition of voltage-gated Ca\textsuperscript{2+} channels induced by CB\textsubscript{1} receptor activation (Niehaus et al, 2007). Therefore, this provides a new avenue for modulation of the endocannabinoid system. Stimulation of CB\textsubscript{1} receptors activates G\textsubscript{i/o} proteins, affecting multiple downstream signalling events including activation of inwardly rectifying potassium channels traditionally measured with biochemical assays or relatively invasive electrophysiological techniques.

Aims. To determine whether measurement of membrane potential in intact cells is a suitable assay for examining changes in CB\textsubscript{1} signalling in a cell line endogenously expressing both G-protein coupled inwardly rectifying potassium (GIRK) channels and CRIP\textsubscript{1a}. Secondly, to explore the effects of CRIP\textsubscript{1a} knockdown on modulation of CB\textsubscript{1} receptor mediated activation of GIRK channels.

Methods. AtT20 cells stably expressing CB\textsubscript{1} were grown in 96-well microplates and serum starved overnight. Cells were incubated with a proprietary membrane potential-sensitive dye (Molecular Devices, Blue Dye) and continuous fluorescence reading obtained using a FLEX Station Microplate Reader.

Results. CB\textsubscript{1} receptor agonist WIN55212-2 activated CB\textsubscript{1}, pEC50 6.8±0.1 (n=5) producing a maximum change in fluorescence of 30.54±2.31% compared to CP55940, pEC50 7.1±0.1 (n=6) which showed a maximum change in fluorescence of 27.09±1.34%. Anandamide as expected showed both a decrease in potency with a pEC50 of 5.7±0.2 (n=5) and efficacy with a maximum change in fluorescence of 20.5±1.0%. In addition, siRNA-induced CRIP\textsubscript{1a} knockdown significantly increased anandamide-induced GIRK channel activation (n=4, P<0.05).

Discussion. Given that CRIP\textsubscript{1a} knockdown does not have any effect on GIRK channel activation in response to WIN55212-2 or CP55940, suggests that CRIP\textsubscript{1a} modulates CB\textsubscript{1} receptor signalling in a ligand-specific manner.

Mechanical hyperalgesia, but not alldynia, is sustained through α9-nicotinic ACh-receptor activity.
Sarasa A Mohammadi1 & Macdonald J Christie1. Depart of Pharmacol, Univ of Sydney1, Sydney, NSW

Introduction. Chronic pain is poorly managed pharmacologically. Conotoxins from marine cone snails are a source of potential analogues. Vc1.1 is an α-conotoxin producing effective, sustained relief of mechanical allodynia and hyperalgesia in rodent models of neuropathic pain. Two molecular targets could mediate these actions. Vc1.1 potently and specifically inhibits nAChRs containing α9 subunits. However, antagonism of the α9-nAChRs has been suggested to be neither sufficient nor necessary for pain relief (Nevin et al, 2007). Vc1.1 also inhibits N-type Ca2+ channel currents in a GABA_B receptor-dependent manner (Callaghan & Adams, 2010), and in vivo, GABA_B antagonists reverse acute Vc1.1 anti-allodynia (Klimis et al, 2011).
Aims. To determine whether or not deletion of α9-nAChRs affects the development and persistence of chronic pain in an animal model.
Methods. Several sciatic nerve injury models were tested in α9 nAChR-knockout (KO) and wild-type (WT) mice. Differences in mechanical allodynia (von Frey and incapacitance tests) and hyperalgesia (paw pressure test) were assessed.
Results: KO mice develop mechanical allodynia that is indistinguishable from WTs, which persists for at least 3 weeks. Mechanical hyperalgesia also develops in the KO within 1 week (KO: 61±67% of pre-surgical response, n=6; WT: 45±55%, n=6, p<0.001, Bonferroni one-way ANOVA) but greater recovery is observed by the second week post surgery (KO: 89±55 % of pre-surgical response, n=6; WT: 48±45%, n=6, p<0.01, Bonferroni one-way ANOVA).
Discussion. The results show that mechanical hyperalgesia is less persistent when the α9-nAChR is deleted but mechanical allodynia is unaffected. Perhaps, whilst the acute anti-allodynic effects of Vc1.1 do not involve the α9-nAChR, sustained relief of mechanical hyperalgesia may be achieved through α9-nAChR inhibition.


Ω Omega-conotoxins CVID and CVIE and two analogues display age-sensitive differences in biophysical properties in sensory neurons
Swetha S. Murali1, Ian A. Napier1, Richard J. Lewis2, Paul F. Alewood3, MacDonald J. Christie1, Discipline of Pharmacology, University of Sydney1, Sydney, NSW, Institute for Molecular Bioscience, University of Queensland2, Brisbane, QLD

Introduction: Omega-conotoxins that selectively block N-type calcium channels are potential new therapeutics for the treating pain. We were interested in two omega-conotoxins, CVID and CVIE, which were found to have different effects in neonatal and adult rat dorsal root ganglion (DRG) neurons. Previous studies have shown that the reversibility of omega-conotoxins is dependent on differential expression of calcium channel subunits, which could potentially vary during development.
Aims: To measure the concentration-response and reversibility of omega-conotoxins CVIE and CVID in neonatal and adult DRG neurons.
Methods: Whole-cell patch clamp recordings of VGCCs was performed in isolated DRG neurons from adult (>6 weeks) and neonatal (4-12 days) male rats.
Results: Near maximal concentrations of CVID (100 nM) and CVIE (300 nM) inhibited the total I_Ca in all neurons (n=32) by 49 ± 4 %, with no significant difference in maximal inhibition between CVID and CVIE. In DRG neurons from adult rats, complete recovery was seen from 300 nM CVIE in all neurons (n=9). Recovery from CVID was variable, with no recovery in 4 cells, partial recovery in 1 and complete recovery in 6 out of 11 cells. In DRG neurons from neonatal rats, recovery from CVIE block was tested in two cells, with complete recovery in one and partial recovery in the other. There was no recovery from CVID in 7 neurons, and partial recovery in 1. The recovery from CVID block was significantly different in adult and neonatal neurons (P<0.0001).
Conclusion: Recovery from omega-conotoxin block is different in neonatal and rat DRG neurons.
Ciguatoxin-induced cold allodynia is an acquired peripheral channelopathy involving preferential activation of TRPA1-expressing nociceptors

Irina Vetter 1, Filip Touska 2, Rachel Hinsbey 3, Simon Sattler 2, Angelika Lampert 2, Anastasia Sharov 4, Lindon Collins4, Peter Cabot4, John Wood3, Victorie Vlachova5, Peter Reeh2, Richard Lewis1, Katharina Zimmermann2.

1Institute for Molecular Bioscience, University of Queensland, St Lucia, QLD, Australia, 2Department of Physiology and Pathophysiology, University of Erlangen, Erlangen, Germany, 3Wolfson Institute for Biomedical Research, University College London, London, UK, 4School of Pharmacy, University of Queensland, St Lucia, QLD, Australia, 5Department of Cellular Neurophysiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Introduction. Ciguatera is a common form of fish intoxication that typically involves several painful neuropathy-like syndromes including cold alldonyia, arthralgias and myalgias. At the molecular level, ciguatoxin (CTX) is the most potent known activator of voltage-gated sodium channels. Despite the exquisite sensitivity of neurons to CTX, the precise sensory neuronal populations activated by CTX have not been determined. Specifically, it remains uncertain how CTX causes pain or how it modulate sensory inputs by altering the activity of different types of dorsal root ganglion neurons. The neuronal population activated by low concentrations of CTX may be responsible for the persistent cold-allodynia caused by ciguatera.

Aims. We sought to identify the sensory neuronal populations mediating these symptoms and to elucidate the cellular and molecular basis of ciguatoxin-induced cold allodynia.

Methods. We assessed the effects of CTX on peripheral neurons using a range of techniques including fluorescent calcium imaging of DRG neurons, animal behaviour and single fibre recordings.

Results. We show that intraplantar injection of P-CTX-1 elicits cold allodynia in mice by targeting specific unmyelinated and myelinated primary sensory neurons. These include both tetrodotoxin-resistant, TRPA1-expressing peptidergic C-fibres and tetrodotoxin-sensitive A-fibres. P-CTX-1 does not directly open heterologously expressed TRPA1, but when co-expressed with Nav channels, sodium channel activation by P-CTX-1 is sufficient to drive TRPA1-dependent calcium influx that is responsible for the development of cold allodynia, as evidenced by a large reduction of excitatory effect of P-CTX-1 on TRPA1-deficient nociceptive C-fibres and of ciguatoxin-induced cold allodynia in TRPA1-null mutant mice.

Discussion. These findings establish a peripheral site of action for ciguatoxins and reveal that altered excitability of peripheral sensory neurons can be sufficient for the development of cold allodynia.
The influence of surgery-induced inflammation and isoflurane anaesthesia on postoperative cognitive outcome
Catherine M Wood1, Colin F Royse1, Jennifer K Callaway1. Department of Pharmacology, University of Melbourne1, Parkville, VIC

Introduction. The pathogenesis of post-operative cognitive dysfunction remains unclear, however, studies in young and aged animals suggest that anaesthesia and/or surgically-induced inflammation can affect cognitive outcome.

Aim. Using a rat model, we aim to investigate the role of isoflurane anaesthesia alone or with surgically-induced inflammation.

Methods. Male Sprague Dawley rats were subjected to isoflurane (n=9, 4h, 1.8% in 100% O2). Controls were subjected to 10 min of O2 (n=14). Laparotomy was performed in another group of isoflurane-treated animals (n=9), and the wound left open for 10min then sutured. Eight days after isoflurane exposure, cognition was tested in a fear conditioning paradigm. Rats were placed in a chamber in which they received a foot shock (1mA, 1s duration). When returned to the chamber the percentage of time spent in freezing behaviour was recorded as a measure of memory for the shock previously experienced in that chamber. One day after fear conditioning, rats were deeply anaesthetised and transcardially perfused. Hippocampal tissue was collected and processed for cytokine analysis (Bio-Plex™).

Results. Rats exposed to isoflurane showed significantly decreased freezing behaviour compared to no-anaesthesia controls, indicating a memory impairment (25.4±9.4% vs 66.2±8.9%, P<0.01). Rats in the isoflurane plus surgery group also had impaired memory (35.3±7.5%) but this was not worse than isoflurane alone (P>0.05). Isoflurane exposure was associated with increases in pro-inflammatory cytokines in the hippocampus including IL-6 and TNF-α (P<0.05) compared to controls and isoflurane plus surgery significantly increased TNF-α in the hippocampus (P<0.05).

Discussion. The finding suggests isoflurane exposure impairs memory, while additional surgical trauma does not worsen memory. Increased inflammatory cytokines in the hippocampus may be a possible mechanism behind memory impairment.

A rapid and sensitive LC-MS method for the quantification of nilotinib in human plasma.
Daniel T Barratt1, Joel D Colvill1, Deborah L White2, Timothy P Hughes2 & Andrew A Somogyi1. Disc of Pharmacology, Univ of Adelaide1, Adelaide, SA; Dept of Haematology, SA Pathology2, Adelaide, SA.

Introduction. Nilotinib is a potent second-generation tyrosine kinase inhibitor used in chronic myeloid leukaemia treatment. Nilotinib plasma concentrations vary significantly between patients for a given dose, and sensitive, reproducible and efficient assays for quantifying nilotinib in patient plasma are required to support current clinical studies of optimal personalised nilotinib dosing.

Aims. To develop a rapid and sensitive LC-MS method for quantifying nilotinib in plasma, and demonstrate its application in chronic myeloid leukaemia patient samples.

Methods. Plasma samples (250 μL) were prepared by addition of d3-nilotinib internal standard, protein precipitation and centrifugation. 5 μL of supernatant was resolved with a C18 LUNA column (5 μM, 150 x 2.0 mm i.d., Phenomenex) using mobile phase (55:45 4 mM ammonium formate formate pH 3.2 : 0.1% formic acid in acetonitrile) at a flow rate of 0.15 mL/min. Detection was carried out on a LCMS-2010A Mass Spectrometer (Shimadzu) at m/z ratios of 530.10 and 533.10 for d0- and d3-nilotinib, respectively. Sample run time was 5 minutes. Steady-state trough plasma nilotinib concentrations (morning pre-dose on day 8 of treatment) were quantified in 57 chronic myeloid leukaemia patients receiving 300 mg nilotinib twice daily.

Results. Standard curves were linear from 20-5000 ng/mL (r2>0.998, n=6). Intra- (n=6) and inter-day (n=6) inaccuracy and imprecision for quality controls samples (60, 30 and 1750 ng/mL) was within 14%, and within 10% for the lower limit of quantification (20 ng/mL). Nilotinib plasma concentrations in clinical samples ranged from 37 to 3470 ng/mL (median=810 ng/mL, 63% coefficient of variation).

Discussion. The LC-MS method developed has sufficient sensitivity to quantify clinically relevant nilotinib plasma concentrations within a short run time, and without the need for solid phase extraction, drying and reconstitution, or tandem mass spectrometry. The reported method is currently supporting multiple clinical trials of nilotinib in the treatment of chronic myeloid leukaemia.
Glucosidation and glucuronidation of mycophenolic acid (MPA) by UDP-glucuronosyltransferase (UGT) 1A and 2B sub-family enzymes

Nuy Chau1, Peter I. Mackenzie1 & John O. Miners1. Department of Clinical Pharmacology, Flinders University1, Adelaide, SA.

Introduction. Mycophenolic acid (MPA) is a lipophilic drug used in immunosuppressive therapy. It is primarily glucuronidated by UGT1A9, but the role of glucosidation remains unclear. In vitro studies have shown MPA glucosidation occurs in humans.

Aim. To characterise the comparative kinetics of MPA glucosidation and glucuronidation in human liver microsomes (HLMs) and to identify the human UGT enzyme(s) capable of glucosidating MPA.

Methods. Formation of MPA phenolic glucuronide (MPAGlcUA), MPA phenolic glucoside (MPAGlc), MPA acyl glucuronide (AcMPAGlcUA) and MPA acyl glucoside (AcMPAGlc) from incubations with microsomes from four livers (HLM) and recombinant UGT enzymes (expressed in HEK293 cells) supplemented with the appropriate cofactor (UDP-GlcUA or UDP-Glc) was quantified by HPLC with UV detection.

Results. MPAGlcUA, AcMPAGlcUA and AcMPAGlc formation by HLM followed Michaelis-Menten kinetics with average clearances (CL_int) of 45.5±9.8μL/min/mg, 8.4±1.3μL/min/mg and 0.73±0.08μL/min/mg, respectively. The presence of both UDP-GlcUA and UDP-Glc significantly decreased the CL_int for MPAGlcUA (0.66±0.24μL/min/mg) and abolished AcMPAGlc formation. Screening of recombinant human UGT enzymes indicated that UGT1A9 was the main enzyme that catalyzed both the glucuronidation and glucosidation of MPA. MPAGlcUA was formed only by UGT1A family enzymes. In comparison, MPAGlc was formed by both the UGT1A family enzymes and UGT2B7. UGT1A10 and UGT2B7 were the main enzymes responsible for the formation of AcMPAGlcUA. UGT2B7 was the only enzyme found to convert MPA to AcMPAGlc.

Conclusion. Like morphine, the glucosidation and glucuronidation of MPA occur as parallel metabolic pathways but both reactions were catalysed by several enzymes from the UGT1A family.
Cediranib and erlotinib are potent inhibitors of human solute carrier transporters
Rosie A Johnston, Fanfan Zhou, Tristan Rawling and Michael Murray. Pharmacogenomics and Drug Development Laboratory, Faculty of Pharmacy, Univ of Sydney, Sydney, NSW.

Introduction. Members of the solute carrier family of transporters regulate the cellular influx of several drugs. OATP1A2 (SLCO1A2) and OATP1B3 (SLCO1B3) participate in the disposition of a number of anticancer drugs, including the tyrosine kinase inhibitor imatinib (Hu et al, 2008).

Aims. To evaluate the impact of tyrosine kinase inhibitors on the uptake of a prototypical substrate in cells overexpressing SLC transporters.

Methods. HEK293 cells were transfected with transporter cDNAs, including organic anion transporters (OAT1-4), organic anion transporting polypeptides (OATP1A2, 1B1, 1B3 and 2B1) and organic cation transporters (OCT1-3). Tyrosine kinase inhibitors (10 μmol/L) were tested for the capacity to inhibit the uptake of a specific transporter-mediated radiolabelled substrate into these cells. Half maximal inhibitory concentrations (IC50) of potent inhibitors were determined.

Results. Of the 13 clinically relevant tyrosine kinase inhibitors tested, 11 effectively inhibited substrate uptake in some transporters. Two of these interactions had an IC50 in the nanomolar range: cediranib (IC50 of 30.6 nmol/L) and erlotinib (IC50 of 19.7 nmol/L) against OATP1A2 and OATP2B1, respectively. Because the maximum plasma concentration of cediranib is 0.235 μmol/L (Fox et al, 2010), this drug has the potential to inhibit OATP1A2-dependent drug transport at clinically relevant concentrations.

Discussion. The expression of several solute carrier transporters is modulated in cancer cells. Individuals with altered expression of these proteins may have different antitumour efficacy with tyrosine kinase inhibitors. Some of these agents may also elicit drug-drug interactions during therapy.

Liver fibrosis as the major determinant of the altered hepatic uptake of taurocholate in liver diseases

Peng Li1,2, Qian Zhang1,2, Linda M Fletcher3, Darrell HG Crawford3, Michael Weiss4, Michael S Roberts1,2. School of Pharmacy and Medical Sciences, University of South Australia and the Basil Hetzel Institute for Medical Research, the Queen Elizabeth Hospital1, Adelaide, SA; School of Medicine, The University of Queensland2, Brisbane, QLD; Department of Gastroenterology and Hepatology, School of Medicine, University of Queensland2, Brisbane, QLD; Department of Pharmacology, Martin Luther University Halle-Wittenberg4, Halle, Germany.

Introduction. A number of substrates are taken up into the liver by drug transporters on sinusoidal membrane. In chronic liver diseases, the uptake of these substrates can be affected by liver transporter expression and by liver fibrosis that can arise in the space of Disse.

Aims. To examine the relative contribution of altered drug transport expression and liver fibrosis to the altered hepatic disposition.

Methods. The hepatic disposition of taurocholate was studied using in situ perfused rat liver (IPRL) in rats with a number of different liver diseases, including nonalcoholic steatohepatitis (NASH), right heart failure (RHF) and cirrhosis. The fibrosis index (FI) was used to quantify the liver fibrosis, while mRNA and protein expression of sodium-dependent taurocholate co-transporting protein (Ntcp) was determined by RT-PCR and Western Blot, respectively, to evaluate transporter function.

Results and Discussion. A good linear relationship was find between the hepatic uptake of taurocholate and FI in both the control and rat models of different liver diseases (r²=0.858). However, further stepwise regression analysis with both FI and transporter mRNA/protein expression did not significantly improve the prediction of taurocholate uptake in the liver diseases. Moreover, the good linear relationship between the reduced hepatic extraction of taurocholate and FI was also observed (r²=0.780). In conclusion, the liver fibrosis in the space of Disse is major determinant of the reduced hepatic uptake of taurocholate in liver diseases.

In vivo bio-distribution of water-dispersible CdTe/CdS quantum dots following intravenous injection

Xiaowen Li1, Xin Liu1, Jeffrey Grice1, Zhiping Xu2, Michael S Roberts1,3. Therapeutics Research Centre, School of Medicine, The University of Queensland1, Brisbane, QLD; ARC Centre of Excellence for Functional Nanomaterials, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland2, Brisbane, QLD; School of Pharmacy and Medical Science, University of South Australia3, Adelaide, SA.

Introduction. Quantum dots (QDs) are potentially useful in tumour diagnosis, as bio-indicators, and in drug delivery.

Aims. To investigate the bio-distribution of water-dispersible QDs in mice following intravenous injection.

Methods. Water dispersible cadmium telluride (CdTe) QDs (~ 3.5 nm) were synthesised in aqueous solution, purified, characterised and administered to a total of 21 mice at a dose of 0.02 nmol/g by tail vein injection. At each time point (0, 5 min, 30 min, 1 h, 2 h, 8 h and 24 h), whole body fluorescence imaging (IVIS spectrum, Xenogen) was performed on 3 mice which were then sacrificed. The Cd content of heart, spleen, kidney, liver, intestine, lung, brain and blood was determined by ICP-MS after digestion with nitric acid and appropriate dilution.

Results. The QDs organ and blood concentration – time distribution profiles in organs and blood obtained using in vivo fluorescence and ICP-MS were similar but the ICP-MS was more sensitive. After intravenous injection, the QDs quickly distributed from the blood into organs with a volume of distribution of 0.185 ml/g. The clearance and elimination half-life were determined to be 0.009416 ml/h/g and 13.6 h, respectively. The spleen and liver were the main target organs for QD uptake and, in these organs, peak concentrations were reached at 1 and 2 hours, respectively. Smaller amounts of QDs were detected in heart, lung, kidney, intestine. Very low levels of QDs were found in the brain.

Discussion. Water-dispersible small QDs showed rapid uptake into various organs after intravenous dosing. Highest concentration of QDs were found in the reticuloendothelial system organs of the liver and spleen.
Effect of liver endothelial cell defenestration on hepatic insulin and glucose uptake
Mashani Mohamad1,2, Victoria C Cogger1, David G Le Couteur1. Centre for Education and Research on Ageing and ANZAC Medical Research Institute, University of Sydney and Concord RG Hospital1, Concord, NSW; Faculty of Pharmacy, Universiti Teknologi MARA2, Bandar Puncak Alam, MALAYSIA

Introduction. Fenestrations in the liver sinusoidal endothelium facilitate the transfer of substrates from blood to hepatocytes. A recent study suggested that the hepatic sinusoidal endothelium has a role in the pathogenesis of hepatic insulin resistance and the metabolic syndrome. Therefore, we proposed that a decrease in fenestrations (defenestration) impedes the transfer of insulin and glucose across the hepatic sinusoidal endothelium, thus contributing to hepatic insulin resistance.

Aims. To investigate the disposition of insulin and glucose in liver and how this is influenced by poloxamer 407 (P407), a synthetic surfactant that causes defenestration and hyperlipidemia.

Methods. Multiple indicator dilution method was performed in perfused livers of control rats (n=9) and rats injected intraperitoneally with P407, 24h prior to experimentation (1g/kg, n=8). The indicators used were Evans Blue (vascular marker), 3H-sucrose (extracellular marker) and either 14C-glucose or 14C-insulin. Outflow samples were analyzed for absorbance at 620 nm and radioactivity using liquid scintillation counter to determine substrate recovery and volume of distribution.

Results. Animals treated with P407 showed a significant increase in triglyceride and cholesterol levels compared to control (p<0.001), together with a marked defenestration in the liver sinusoidal endothelial cells. The recoveries of both glucose and insulin was reduced in the hyperlipidemic rats, along with significant decreases in volumes of distribution as a fraction of sucrose for both substrates (glucose: 1.54±0.06 control vs 1.10±0.10 P407; insulin: 1.08±0.08 control vs 0.81±0.05 P407, p<0.001).

Discussion. P407 induced defenestration of the sinusoidal endothelium and reduced the recoveries and volumes of distribution of insulin and glucose in the liver. This finding indicates that fenestrations are important in the uptake of insulin and glucose and that defenestration may have a role in hepatic insulin resistance.
Diurnal variation in CYP1A2 activity in individuals of South Asian and European ancestry
Vidya Perera1,2,3, Annette S Gross1,4, Andrew J McLachlan1,2 Faculty of Pharmacy, The University of Sydney, Sydney, NSW, Centre for Research and Education on Ageing, Concord Hospital, Concord, NSW, Department of Pharmacy Practice, Faculty of Pharmacy, SUNY at Buffalo5, Buffalo NY, USA, Clinical Pharmacology Modelling and Simulation, GlaxoSmithKline R&D6, Sydney, NSW

Introduction: Response to some medications can depend on time of administration throughout the day. The cytochrome P450 1A2 (CYP1A2) enzyme demonstrates wide variability which is observed in human population studies.

Aims. This study investigated diurnal variation in CYP1A2 activity in people of South Asian and European ancestry. Methods. CYP1A2 activity was determined using the 4-h paraxanthine/caffeine saliva concentration ratio following a 100-mg oral dose of caffeine in healthy individuals of South Asian (n = 11) and European (n = 12) ancestry. Caffeine was administered in the morning and evening on three separate days.

Results. The index of CYP1A2 activity (mean ± SD) was higher in the morning (0.52 ± 0.17) when compared with evening (0.47 ± 0.17) (n = 23, P < 0.05). When stratified by ethnicity, a difference in CYP1A2 activity was observed between the morning (0.43 ± 0.13) and evening (0.35 ± 0.05) for individuals of South Asian ancestry (P < 0.05), but not in those of European ancestry (0.61 ± 0.15 and 0.56 ± 0.17, respectively). A significantly lower CYP1A2 activity was observed in South Asian participants compared to those of European ancestry in both periods (p < 0.05).

Discussion. This study observed higher CYP1A2 activity in subjects of South Asian but not European ancestry in the morning than in the evening. These results indicate that time of day may be an important consideration when administering CYP1A2 metabolised medications.

Inhibition of human UDP-Glucuronosyltransferase 1A (UGT1A) enzymes by three different Tyrosine Kinase Inhibitors (Lapatinib, Pazopanib and Sorafenib): implications for drug interactions and jaundice
Ganessan Kichenadasse1, Andrew Rowland1, John O Miners1: Clinical Pharmacology, Flinders University1, Adelaide, South Australia.

Introduction. Tyrosine kinase inhibitors (TKIs) are of proven efficacy in the treatment of a number of cancers: e.g. sorafenib for kidney and liver cancers; lapatinib for breast cancer and pazopanib for kidney cancers and sarcoma. Patients receiving TKIs are at risk of drug interactions due to polypharmacy. However, the potential of TKIs to perpetrate drug-drug interactions remains to be fully characterized.

Aims. To characterize the inhibitory effects of lapatinib, pazopanib and sorafenib on the activities of UGT1A sub-family enzymes to assess the likelihood of clinically significant inhibition in vivo.

Methods. Recombinant human UGT1A enzymes and human liver microsomes (HLMs) were utilized as the enzyme sources. Inhibition of recombinant UGT1A enzyme activities was assessed at 4 different TKI concentrations (0.01, 0.1, 1 and 10 μM) using 4-methylumbelliflorone (4MU) as the probe substrate (Uchaipichat et al., 2004). Kinetic experiments for sorafenib inhibition of bilirubin glucuronidation were performed.

Results. Sorafenib (10 μM) inhibited all UGT1A enzymes. IC50 values for UGT1A1, UGT1A7, UGT1A8 and UGT1A9 were < 1 μM. Derived IC50 values were 0.051 μM, 0.034 μM, 0.031 μM and 0.039 μM for wild-type UGT1A1-HLM, UGT1A*28-HLM, wild-type UGT1A1, and UGT1A1*6, respectively for bilirubin glucuronidation. Pazopanib inhibited UGT1A1, UGT1A7, UGT1A8 and UGT1A9 with Ki values <10 μM. Lapatinib showed similar inhibition of UGT1A1, UGT1A3 and UGT1A9.

Discussion. All the three TKIs (lapatinib, pazopanib and sorafenib) are potent inhibitors of UGT1A1 with Ki values < 2 μM. Current data indicate that there is a potential for the screened TKIs to cause inhibition of UGT1A1 specific substrates like bilirubin. This is consistent with the clinical finding of drug induced jaundice by these TKIs.

**Allometric scaling of antimalarial drugs**

Ganga Senarathna1,2,3, Kevin T Batty1,2. School of Pharmacy1 and Curtin Health Innovation Research Institute2, Curtin University, Bentley, WESTERN AUSTRALIA; Pharmacy Program3, University of Sri Jayewardenepura, SRI LANKA.

Introduction. World Health Organization and pharmacopoeial dosage recommendations for most antimalarial drugs are the same (mg/kg) for children and adults. However, the clearance (L/h/kg) for many drugs is higher in children than in adults and there is recent evidence that children require higher chloroquine doses (mg/kg) compared to adults for optimum clinical outcome (Obua et al, 2008, Moore et al, 2011). Aims. Conduct interspecies allometric scaling of CL and V for selected antimalarial drugs and investigate the interpolation of CL data for dose predictions in children.

Methods. Pharmacokinetic data (CL and V) for antimalarial drugs in healthy and malaria infected species were collated. Regression analysis of log-transformed data was performed to determine the coefficient (a) and exponent (b) for the allometric equation, Y = a×Wb, where Y is the pharmacokinetic parameter and W is body weight. The exponent for CL and the recommended adult dose were used to predict a scaled dose for a 25 kg child.

Results. A paucity of data (<3 species) precluded scaling in malaria infection and restricted the range of drugs investigated. Exponents for CL of quinine, mefloquine, clindamycin, and dihydroartemisinin in healthy species were 0.4, 0.52, 0.63 and 0.8 respectively (r² >0.9) and the exponents for V were 0.88, 0.78, 0.8 and 0.8, respectively (r² >0.9). The predicted child doses for quinine, mefloquine, clindamycin and dihydroartemisinin were 84%, 64%, 46% and 14% higher (mg/kg for 25 kg child) than adult doses.

Discussion. Our data indicate that higher mg/kg doses of some antimalarials are required for children. The doses should be determined for individual drugs, with consideration of altered pharmacokinetic properties in malaria infection, and not based on a universal, fixed exponent.


---

**In vitro characterisation of the ‘albumin effect’ on human liver microsomal olanzapine oxidative metabolism**

Porntipa Korprasertthaworn1, Andrew J McLachlan2, Thomas M Polasek1, John O Miners1, Andrew Rowland1 Dept of Clin Pharm, Flinders University1, Adelaide, SA; Fac of Pharm, University of Sydney2, Sydney, NSW.

Introduction. Olanzapine (OLZ) is an atypical antipsychotic commonly prescribed for the management of schizophrenia and related psychosis. Although there is wide inter-individual variability in OLZ pharmacokinetics and response, the metabolism of OLZ is incompletely characterised and CYP1A2 is thought to play a significantly role. Thus, factors that influence the clearance of OLZ, and subsequently maintenance dose to attain therapeutic steady-state concentration, are poorly understood.

Aims. To investigate the ‘albumin effect’ on the oxidative metabolism of OLZ in human liver microsomes (HLM).

Methods. A UPLC-MS method was developed and validated to quantify OLZ and its three oxidative metabolites (N-demethyl-, hydroxy-, and N-oxide-olanzapine). Assay conditions were validated with respect to incubation time, protein concentration, and reproducibility. The kinetics of OLZ metabolite formation (Km and Vmax) were characterised in the presence and absence of bovine serum albumin (BSA; 2%). Binding of OLZ to incubation components was accounted for in the calculation of kinetic parameters. Whole liver intrinsic clearance (CLint,liver) was predicted using hepatic scaling factors.

Results. In the absence of BSA, OLZ N-demethylation and N-oxidation were best described by the Michaelis-Menten equation. CLint,liver values for these pathways were 4.7 and 1.5 L/h, respectively. The OLZ hydroxylation pathway was best described by the 2-enzyme Michaelis-Menten equation; with a combined CLint,liver of 0.8 L/h. In the absence of BSA, the total CLint,liver for HLM catalysed OLZ oxidative metabolism was 7.0 L/h. Addition of BSA caused a 2-fold increase in total HLM catalysed OLZ metabolism, primarily due to a reduction in Km for the N-demethylation and N-oxidation pathways. The total CLint,liver in the presence of BSA was 13.5 L/h.

Discussion. These data indicate that N-demethylation is the primary oxidative pathway for OLZ. The ‘albumin effect’ increases the N-demethylation and N-oxidation of OLZ and results in a 2-fold increase in predicted whole liver intrinsic clearance.
Combination therapy with oxycodone and zoledronic acid in inflammatory arthritis: The role of cytokines in acute and chronic inflammation.
Waltraud Binder, Jignya H Patel. School of Medical Sciences, University of NSW, Sydney 2052, Australia (introduced by Nicole Jones, School of Medical Sciences, University of NSW, Sydney 2052, Australia).

Introduction: Cytokines that are abundantly produced in inflamed rheumatoid synovial fluid, such as tumour necrosis factor α (TNF-α), interleukin-1β (IL-1β), and IL-17, play crucial roles in the pathophysiology of rheumatoid arthritis (RA). Bisphosphonates inhibit bone destruction and are shown to increase bone density in animal models of RA while opioids are used as adjunct drugs for analgesia and are also known to have anti-inflammatory effects.

Aim: To investigate the role of inflammatory cytokines in the mechanism of action of oxycodone and zoledronic acid (ZA) in attenuating acute and chronic inflammation.

Methods: Dark Agouti (DA) rats were induced with adjuvant arthritis by inoculation with Freund’s complete adjuvant either into the right hind paw or the base of the tail to induce mono and poly arthritis respectively. Oxycodone and ZA were administered at different doses and time intervals. Immunohistochemistry was utilised to determine the localisation of inflammatory cytokines in rat ankle joints.

Results: Combination therapy significantly reduced bone damage and showed analgesic effects. Oxycodone and ZA in combination proved to be very effective in combating arthritis by reducing inflammation and pain as well as inhibiting bone damage. Individual treatment reduced arthritic severity by 81% and 63% at an optimum dose of oxycodone 5mg/kg and ZA 3µg/kg respectively. Combination therapy was effective in reducing arthritic severity at a lower dose than individual therapy; the optimum dose combination was oxycodone 5mg/kg and ZA 1µg/kg. Paw pressure thresholds significantly decreased in treated arthritic rats (P<0.05), while control animals showed significant hyperalgesia. The treatment also reduced the levels of the proinflammatory cytokines TNF-α, IL-1β and IL-17 in the tibial-tarsal joint as compared to controls. Conclusion: The results suggest that combining oxycodone and ZA has a potential therapeutic relevance in the treatment of arthritis.


Choice of contractile agonist influences dilator efficacy in small airways in mouse lung slices
Meaghan FitzPatrick, James Esposito, Chantal Donovan, Mirjam Simoons and Jane. E. Bourke. Dept of Pharmacology, University of Melbourne, Parkville, VICTORIA.

Introduction. Altered reactivity of small airways may contribute to airway hyperresponsiveness and reduced β2-adrenoceptor sensitivity in severe asthma. It is therefore important to characterise small airway contraction to diverse agonists, such as methacholine (MCh) and endothelin-1 (Et-1); the latter being markedly increased in treatment-resistant asthma (Pegorier et al., 2007). These constrictors can then be used to assess the relative efficacies of bronchodilator therapies.

Aim. To assess the influence of contractile stimuli, MCh and Et-1, on small airway relaxation to salbutamol (SAL), prostaglandin-E2 (PGE2) and novel bronchodilator rosiglitazone (RGZ).

Methods. Changes in small airway lumen area were measured in lung slices (150 µM) from 6-8 week old male Balb/C mice. After characterising contraction to MCh and Et-1, dilator responses were assessed at varying levels of pre-contraction.

Results. Et-1 was 19-fold more potent than MCh (pEC50 8.5 ± 0.1, 7.1 ± 0.1 respectively, p < 0.05), and both reduced airway lumen area by up to 50%. The rank order of dilator potency was PGE2 > SAL > RGZ, where maximal relaxation of ~75% was achieved by RGZ against both constrictors. PGE2 and SAL were markedly less effective than RGZ in relaxing Et-1 pre-contraction, and β2-mediated relaxation completely abolished in maximally contracted airways.

Discussion. Et-1 is a potent constrictor of mouse small airways. SAL was the least effective of the dilators tested. Although PGE2 was the most potent, RGZ was as effective as PGE2 against MCh, and more effective against Et-1. This study emphasises the need to explore the clinical potential of novel dilators against multiple contractile agonists implicated in human asthma.

**Investigating the role of PTEN in airway epithelial inflammation and remodelling in Chronic Obstructive Pulmonary Disease (COPD)**

Amanda Vannitamby, Huei Jiunn Seow, Ross Vlahos. Dept of Pharmacol, Univ of Melbourne, Parkville, VIC.

Introduction. COPD is characterised by chronic airway inflammation that compromises the integrity of the airway epithelium. PTEN, which acts as a negative regulator of multiple signalling pathways, has shown to be reduced in COPD airway epithelial cells. However, its implications on epithelial inflammation and remodelling in COPD have not been investigated.

Aims. To develop an efficient method that reduces PTEN expression in human bronchial epithelial (Beas-2B) cells, using siRNA and determine whether this has a functional effect on the expression of inflammatory mediators, such as Interleukin-8 (IL-8) and Serum Amyloid A (SAA), initiated by Toll-like Receptor (TLR) signalling pathways.

Methods. To reduce PTEN expression, Beas-2B cells were cultured, harvested and resuspended in low serum optiMEM media. Using a 6-well format NeoFx siport (Ambion) transfection reagent was used to reverse transfected cells with PTEN siRNA (Life Technologies), in accordance with the manufacturer’s instructions. Cells were transfected with Control siRNA as control. To determine whether the loss of PTEN enhanced inflammatory markers, cells were stimulated with 100ng/mL Lipopolysaccharide (LPS) and treated with 10^{-7}M Budesonide (BUD). The cell pellet was collected and stored at 3h and 48h post treatment.

Results. Using Q-PCR (Life Technologies), a 70% reduction (n=6, p<0.05) in PTEN mRNA levels was achieved. Western blot analysis measured PTEN and p-Akt protein levels. A 50% reduction (n=2) in PTEN protein was observed, which was associated with elevated p-Akt at baseline after LPS stimulation (n=2). Reduced PTEN expression did not alter gene expression of the selected inflammatory mediators.

Discussion. These data indicate that in this animal model of cigarette smoke and influenza A (H3N1) infection, the anti-oxidants ebselen and apocynin do not reduce acute exacerbations of CS-induced lung inflammation induced by influenza A infection.
Effectiveness of a blended learning approach in delivering advanced drug delivery systems to third year pharmacy students at Curtin University

Yan Chen1, Kevin K S Tan1, Victor T G Chuang1, Jennifer C Dolzadelli1. School of Pharmacy, Curtin Health Innovation Research Institute, Curtin University1, Perth, WESTERN AUSTRALIA.

Introduction. In order to effectively deliver advanced pharmaceutical technology topics to enhance students’ knowledge, a blended learning approach that comprised lectures, practical and workshop sessions was designed and piloted as part of the teaching program for third year Pharmacy students at Curtin University.

Aims. The aim of the project was to evaluate the effectiveness of the blended learning approach in enhancing students’ understanding and knowledge and to determine the level of its acceptance by students.

Methods. Students were given three standard lectures on advanced drug delivery systems, followed by a 3 hour practical session and a 2 hour workshop. For each practical session, students were divided into groups undertaking preparation and characterisation of nanoparticles, liposomes and microcapsules. Prior to the practical session, students completed a multiple choice question (MCQ)-based test to assess their knowledge of the topic. Follow-up workshops were conducted after the practical session. The workshops included facilitated discussion amongst students and the whole class, group poster work, a post-laboratory MCQ test and a written evaluation of the DDS sessions.

Results. The average score for the pre-laboratory test was 66.4±0.7% (n = 107) and for the post-laboratory assessment using the same set of questions was 82.1±0.9% (n = 107) with most scoring perfect marks. The average score for a new set of 5 questions was 78.7±1.0% (n = 107). In the evaluation, most students indicated that this learning method provided them with a better appreciation of the theory and principles of drug delivery and facilitated their learning.

Discussion. The feedback from students suggests the blended learning approach is effective in optimising the students’ overall learning process and improves student understanding of physical pharmacy theory and its application. Students’ performance in assessment was shown to have improved as a result.
Quality assurance exercise for assessing basic compounding skills of pharmacy students

Jennifer C Dolzadelli¹, Michelle Appleton¹, Richard W Parsons¹. School of Pharmacy, Curtin Health Innovation Research Institute, Curtin University¹, Perth, WA. (introduced by Yan Chen, School of Pharmacy, Curtin University, Perth, WA)

Introduction. Pharmaceutical compounding is a core skill for pharmacists. At Curtin University, pharmacy students compound a range of preparations that are assessed by observation of technique, accuracy of records produced and visual appraisal of product quality, but not analysis to quantify the content of active ingredient. Aims. This study sought to develop compounding laboratory preparations that could be rapidly analysed for content of active ingredient and to investigate the ability of first year pharmacy students, after one semester of training, to accurately prepare simple solutions using weighing, measuring and calculation skills.

Methods. Lignocaine Hydrochloride was identified as a suitable test substance. Laboratory students were instructed to prepare two solutions of Lignocaine HCl 0.025%, one from the drug powder involving the preparation of an aliquot and the other a dilution of a concentrated solution. The solutions were analysed by uv spectrophotometry.

Results. A concentration of 0.025% ± 10% was considered acceptable. Only 47 of 94 students (50.0%) who completed the aliquot solution produced solutions of acceptable concentration. Five (5.3%) students had miscalculated, leaving 42 (44.7%) with deficiencies in weighing and/or measuring skills. 65 of 93 students (69.9%) completed the dilution exercise with acceptable accuracy.

Discussion. This simple exercise demonstrated the inability of a significant proportion of students to accurately compound simple solutions, reflecting the findings of Kadi and co-workers (2005). Deficiencies in students’ weighing and measuring skills were revealed. A greater proportion of students were able to accurately perform the dilution task than the more complicated aliquot solution. The potency analysis provides a powerful teaching tool, via prompt feedback, to encourage students to reflect on, and improve their techniques. This exercise can be employed as a quality assurance measure at any point during pharmaceutical compounding training.

**541**

**What really makes students ‘work ready’ – what are pharmacy students’ and their preceptors’ considerations?**

Dr Jasmina Fejzic¹, Prof Michelle Barker². School of Pharmacy, Gold Coast; Griffith Health Institute (RCCPDI)¹, Griffith University, Brisbane, QLD; Department of International Business and Asian Studies and GIHE², Griffith University, Brisbane, QLD.

Introduction. Comprehensive, first-hand understanding of the concept of pharmacy students’ ‘work readiness’ is vital for academic educators, pharmacy preceptors, and pharmacy students. This can enable academic institutions to provide pharmacy graduates with informed learning strategies that will result in an optimal set of necessary graduate attributes, enabling them to thrive in the increasingly competitive pharmacy market.

Aims. To explore understanding of the concept of ‘work readiness’ amongst the pharmacy students and their preceptors in order to inform future teaching initiatives, particularly in the area of work-integrated learning.

Methods. Community pharmacy preceptors (92) were visited during regular 4th year placement visits by the Griffith University School of Pharmacy Placements Team (two Placements Coordinators, an Associate Lecturer, and the Course Convenor). Preceptors were asked what they believed, in general, constituted ‘work readiness’ in pharmacy interns and their responses were recorded in writing by the visiting Placements Team members. Seventy-one Pharmacy students were asked to reflect and elaborate on what they believed made them ‘work ready’. This survey took place in the lecture, was voluntary and anonymous, and the answers were provided in writing.

Results. In addition to the importance of students’ basic clinical and practice knowledge for their work readiness, the Preceptors’ responses particularly emphasised the value of ‘soft’, transferrable skills in pharmacy graduates (e.g. team work, awareness of workplace dynamic, ‘good personality’, hardworking enthusiastic attitude), and believed that these skills could not always be ‘learnt at university’ but instead needed to be gained through experience or are sometimes simply individual student’s innate traits. Students’ responses were similar to the Preceptor ones, also further emphasising the value of good communication skills, accepting constructive criticism, friendliness, personality, and willingness to learn.

Discussion. Our findings have major implications for optimisation of our Pharmacy curricula, in particular design of their work-integrated and career development components.

**542**

**Competency standards come to life for UTAS Bachelor of Pharmacy undergraduates**

Rose Nash¹, Gregory Peterson², Natalie Brown³, Shane Jackson⁴. School of Pharmacy, Univ of Tasmania¹², Hobart, TAS, TILT, Univ of Tasmania³, Hobart, TAS.

Introduction. Pharmacists use competency standards (CS) [1-2] to attain their right to practice and ensure they continue to review their practice for life. Currently the CS are not well articulated to students in the UTAS BPharm undergraduate curriculum.

Aims. Establish current student awareness, understanding and use of the CS. Provide undergraduate students input in the review and development of their curriculum.

Methods. Using mixed methods UTAS Pharmacy students were surveyed.

Results. A student survey revealed that students do see the CS as important to their studies and assumed they were already considered in designing their curriculum.

<table>
<thead>
<tr>
<th>Question</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you know what the CS are?</td>
<td>93%</td>
<td>14%</td>
<td>89%</td>
<td>93%</td>
</tr>
<tr>
<td>Do you think CS are relevant to you now?</td>
<td>65%</td>
<td>65%</td>
<td>65%</td>
<td>65%</td>
</tr>
<tr>
<td>Do you think CS will be relevant to you in the future?</td>
<td>87%</td>
<td>87%</td>
<td>87%</td>
<td>87%</td>
</tr>
<tr>
<td>Do you refer to the CS to chart your own progress currently?</td>
<td>12%</td>
<td>12%</td>
<td>12%</td>
<td>12%</td>
</tr>
</tbody>
</table>

*P < 0.0001; Students in the current 2nd year BPharm cohort have not received anything on CS in the curriculum.

Discussion. The CS will be further articulated in the UTAS BPharm degree through the development of a validated tool that will assist staff in the review and design of their current units. This process will lead to increased discussion between staff, assist with accreditation requirements and improve benchmarking opportunities with other Schools nationally and internationally.

2. Advanced Pharmacy Practice Framework Steering Committee, Professional Practice Profile for Initial Registration as a Pharmacist. 2011.
The UTAS pharmacy students' road map
Rose Nash1, Gregory Peterson2, Natalie Brown3, Shane Jackson4. School of Pharmacy, Univ of Tasmania1,2,4, Hobart, TAS, TILT, University of Tas3, Hobart, TAS.

Introduction. The School of Pharmacy UTAS has introduced a road map for undergraduate students and staff.
Aims. A flow chart was designed to show students the pre-requisite subjects required for each unit and where their current units would feed into the next semester and year. By mapping the units to the competency standards [1-2] students would be able to have a long-term vision, and recognise the continuity between their first year of study in the Bachelor of Pharmacy (BPharm) through to registration and practice as competent pharmacists.
Methods. In 2012 a flow chart and case study were included in all BPharm unit outlines. Students were surveyed regarding their knowledge and awareness of the competency standards and their acceptance of the flow charts. Themes identified in surveys were further developed in student focus groups.
Results. The flow chart and case study have been embraced and integrated by staff, and welcomed by our students.
All the units in the BPharm degree have been mapped to ascertain if the competency standards had been addressed adequately or if there was unnecessary duplication.

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you feel unit coordinators referred to the competency standards when developing units?</td>
<td>67%</td>
</tr>
<tr>
<td>Do you think it (flow chart) should remain in the unit outline?</td>
<td>67%</td>
</tr>
</tbody>
</table>

Discussion. The course will be reviewed in line with the competency standards to ensure our students are closer to readiness to practice at the successful completion of the BPharm degree.

2. Advanced Pharmacy Practice Framework Steering Committee, Professional Practice Profile for Initial Registration as a Pharmacist. 2011.

Competency of Pharmacy graduates-tool validation and investigation
Melissa J. Austin1, M. Joy Spark1, Christina M. Dennis1. School of Pharmacy and Applied Science, LaTrobe University1, Bendigo, VICTORIA.

Introduction. The pharmacy profession has developed a framework which sets the practice standards for the profession. This framework outlines competency standards (in domains) that professionals within the field should reach. These are expected to be well developed in the undergraduate degree, and throughout the intern year. At this time, there exists no formal tool for assessment of these competencies.
Aims. To validate a questionnaire for use as a tool to assess the competence of pharmacy graduates, and, to investigate the competence of La Trobe University Pharmacy graduates.
Methods. A cross-sectional descriptive study using a quantitative approach was employed. A 56-item questionnaire was sent to employers or preceptors of La Trobe University pharmacy graduates. Data analysis involved descriptive statistics and Rasch analysis through the statistical packages SPSS and RUMM 2030 respectively.
Results and Discussion. Questions for each domain showed high Cronbach's $\alpha$ values (0.843- 0.960), suggesting very good internal consistency of the scales, with some redundancy. High correlating items were identified and deleted from domain, the $\alpha$ values (0.843-0.949) did not significantly change, suggesting their exclusion may not adversely affect overall results. This was confirmed through Rasch analysis, with the changes in PSI values not significantly altering with question deletion. Thus the 56 item questionnaire may be reduced to 50 items without loss of integrity. Overall competency for La Trobe University graduates was found to be high. Total scores for domains of Professional and ethical practice, Review and supply prescribed medicines, Prepare pharmaceutical products, Promote and contribute to optimal use of medicines, Critical analysis, research and education were high, with medians ranging from 3.99 to 4.29. Scores for domains of: communication, collaboration and self-management; leadership and management, and deliver primary and preventive health care were lower with medians ranging from 3.67 to 3.84. Most respondents (94%) said that they would recommend La Trobe graduates to other supervisors or employers. This together with the relatively high competency scores suggests that as a whole, graduates meet the professional practice standards.
Supporting Tasmanian hospital pharmacists to mentor pharmacy students
Catherine J Spiller1, Lisa Crisp1, Marika Castrisios1, School of Pharmacy, Faculty of Health Science, University of Tasmania, TAS.

Introduction. With increased pharmacy student numbers hospital pharmacy departments are being asked to take on more students than in previous decades. For partnerships between the university and these hospitals to remain strong additional support from the university is required.

Aim. The aim of project was to increase clinical placements in Tasmania for Pharmacy students at hospital sites in Tasmania.

Method. Input was sought from pharmacists across the four hospital sites to determine what barriers existed to taking on students for clinical placements. From this, training needs were identified and a two hour, group two accredited CPD session was developed for delivery to pharmacists in three locations across Tasmania.

Results. Evaluation of all trial strategies has been carried out. Preceptors and students have been asked to participate in the evaluation; these will be available for the conference by mid October.

Discussion. Anecdotally the resources and support has been well received by both pharmacists and pharmacy students and we look forward to the results of the evaluations.
Adding creativity to pharmacy practice: Using Bloom’s Taxonomy to develop learning skills in fourth year pharmacy students.
Hung MH Tran1. School of Clinical Sciences (Pharmacy), Queensland University of Technology1, Brisbane, QLD.

Introduction. It is essential for students to have an understanding of factual knowledge for medication therapy management, however students must able to integrate this knowledge and apply it to unfamiliar scenarios. The cognitive process dimensions of the revised Bloom’s Taxonomy states that if a student can function at the creating level, they have mastered the ability to remember and apply knowledge.

Aims. To evaluate graduating pharmacy students’ attitudes of creating and analysing problem-based learning activities in medication therapy management.

Methods. Students were introduced to the concept of Bloom’s Taxonomy in lectures. Structured activities to create problem-based learning problems were completed during tutorials for the disease states covered during the semester to complement traditional knowledge and application tasks. A survey instrument was administered anonymously in September 2012 to graduating pharmacy students at the Queensland University of Technology.

Results. The majority of students (97%) agreed or strongly agreed that creating problem-based learning activities enhanced their learning of medication therapy management and (89%) analysing these created activities enhanced their learning. Thematic analysis of the qualitative data reported that this activity assisted students to integrate previous knowledge and understand the relevance of therapeutic issues in medication therapy management.

Discussion. The systematic and structured integration of activities requiring students to perform skills from the high cognitive processes was accepted by students who perceived that it enhanced their learning of medication therapy management.

Fostering deep learning through collaborative learning and peer assessment: A case study in Pharmacology Teaching and Learning.
Lisa B.G.Tee1, Tin Fei Sim1, Helen Flavell2. School of Pharmacy1, Faculty of Health Sciences2, Curtin University, Perth, Western Australia.

Introduction. Peer assessment and collaborative learning have been shown to improve students understanding of the assessment process (Bloxham & West, 2004). The main challenge for teachers of pharmacology is to transform students’ perceptions from ‘pharmacology equals an extensive amount to know and remember’ to ‘pharmacology equals an interesting and essential subject that enhances competency in both clinical pharmacology and the prescription of medications’.

Aims. Acknowledging that assessment drives learning and that assessment play a major role in shaping student’s perception of learning, collaborative learning and peer assessment were implemented in this study to enhance pharmacology learning.

Methods. The study was conducted in 5 stages. Stage 1: Students worked collaboratively to review lectures. Stage 2: Students formulated questions accompanied with clear marking keys. Stage 3: Moderation of questions. Stage 4: Peer assessment and peer feedback. Stage 5: Evaluation of teaching method.

Results and discussion. In this study involving 218 students, all who have participated in the survey (67 responses) has agreed that the collaborative learning in Stage 1 has enhanced their understanding and deepened their knowledge of Pharmacology. Using the Likert scale, there is 94% agreement that the combination of collaborative learning, exam preparation and peer assessment has helped them developed critical thinking in relation to pharmacology knowledge; 88% and 91% agreement that the activities are engaging and have motivated them to learn, respectively. Students have agreed that the process has helped them remember the content in pharmacology (90% agreement), deepen their knowledge and understanding of pharmacology (89% agreement), increased their confidence in their ability to understand pharmacology and complete the test (89% agreement) and has clarified the assessment criteria (91% agreement). The students suggested the peer assessment to be a closed book test for best result.

Utilization of antihypertensive medication in elderly hospitalized patients

Tariq M. Alhawassi1,2, Beata V. Bajorek3,4, Romano A. Fois1, Lisa G. Pont5. Faculty of Pharmacy, The University of Sydney1, Sydney, NSW; Department of Clinical Pharmacy, King Saud University2, Riyadh, KSA; Graduate School of Health-Pharmacy, The University of Technology Sydney3, Sydney, NSW; Department of Pharmacy and Clinical Pharmacology, Royal North Shore Hospital4, Sydney, NSW; Faculty of Nursing, The University of Sydney5, Sydney, NSW.

Introduction. Hypertension is a common, manageable, chronic risk factor for cardiovascular disease associated with significant morbidity and mortality. Optimal hypertension management is important, yet it is estimated that a significant proportion of elderly patients remain poorly controlled despite wide availability of antihypertensive medications. Understanding the current patterns of antihypertensive utilization is essential for ensuring quality use of medicines and optimising clinical care in elderly hypertensive patients.

Aims. To identify patterns of antihypertensive medication use, including changes to treatment during hospitalisation, in elderly patients.

Methods. A retrospective, cross-sectional study of medical records for elderly patients (age ≥65 years) admitted to a large tertiary teaching hospital in NSW from January to December 2010 was conducted. Medical records were audited to determine the hypertension prevalence and antihypertensive medication use.

Results. Here we present the results for the first 117 patients (mean age: 80.7 years, range: 65.4-99.0, 62.4% female). More than half of the patients (62.4%, n=73) had a documented diagnosis of hypertension and on admission the most commonly prescribed antihypertensives were ACEIs (24.8%, n=18) followed by CCBs (22.7%), ARBs (19.1%), beta-blockers (16%) and diuretics (14%). During hospitalisation 45% (n=33) of patients experienced changes to their antihypertensive regimen. The main changes were cessation of antihypertensive medications, dose reductions or temporary addition of additional antihypertensive agents. The most common reasons documented for these changes were adverse drug reactions (ADRs) (42%, n=14), in particular renal injury and bradycardia, and sub-optimal blood pressure control (33%, n=11).

Discussion. The high prevalence of ADRs associated with antihypertensive medication use in found in this study adds to the challenges of optimal hypertension management in the elderly. Pharmacists need to take this into consideration when optimising blood pressure management during hospitalisation, and need to consider strategies to
Using academic detailing to support nurses’ knowledge and confidence around antipsychotic drugs in dementia
Beate K Antonsen¹, Adam La Caze¹, Michelle Bowden². School of Pharmacy, the University of Queensland¹, Brisbane, QLD; Think Clinical Pharmacy², Brisbane, QLD

Introduction. Antipsychotic agents are frequently prescribed for behavioural and psychiatric symptoms of dementia, despite having modest efficacy and possessing a potential to cause serious harm.
Aims. This project aimed to increase the knowledge and confidence that aged care nurses have about the use of antipsychotic agents in dementia.
Methods. 20 nurses working in five residential care homes in the Brisbane area participated in an academic detailing session about use of antipsychotic agents in dementia. The nurses’ knowledge and confidence around the use of antipsychotic drugs in dementia was measured pre-and-post intervention with a multiple choice quiz and a survey. Certainty based assessment was used to assess nurses’ knowledge regarding the topic and the confidence with which the nurses held this knowledge. The quiz was scored such that respondents that were more often correct with confidence scored higher than those that correct with low confidence or confidently incorrect. The primary assessment of the effectiveness of the academic detailing session was the median change in quiz score. Secondary endpoints included changes in the number of correct answers on the quiz and changes in survey score.
Results. 16 of the 20 nurses who received the education responded to the follow-up quiz and survey, a response rate of 80%. The median quiz score increased from 9.5 points to 21.0 points (p = 0.002) on a scale from –60 to +30 points. The median number of correct answers on the same quiz increased from 7 to 9 out of 10 (p = 0.0002). Respondents reported a high degree of confidence in the survey before and after the academic detailing session.
Discussion. A targeted academic detailing session improved nurses’ knowledge and confidence about the use of antipsychotic drugs in dementia. Most importantly, participants were more likely to be right and confident they were right.

Interprofessional learning: impact on collaboration and attitudes towards health care teams.
Dr Sinthia Bosnic-Anticevich¹, Margaret Williamson², Dr Meg Stuart³, Judith Mackson formerly ², Biljana Cvetkovski¹, Sofia Mavritsakis¹, Gosia Mendrela², Pippa Travers-Mason³, Dr Erica Sainsbury¹, Professor Carol Armour⁴. University of Sydney¹; NPS²; Australian Catholic University³, Woolcock Institute of Medical Research⁴.

Introduction. Health care professionals (HCPs) in primary care are accustomed to working in isolate, hence, for this and many other reasons, collaboration rarely occurs. The Collaborative Asthma Management in the Community (CAMCOM) project involved the development, implementing and evaluating 3 models of interprofessional education on collaborative practice and patient health outcomes. This abstract focuses on the impact of CAMCOM on health care professional collaborative practice.
Aims. To compare the effect of three “interprofessional” educational interventions on attitudes towards collaboration and markers of interprofessional practice.
Methods. HCPs from three general practice networks were recruited into three groups (1, 2, and 3) receiving one of three models of inter professional education (i.e. joint setting group, online group and socio-cultural theory-based group, respectively). HCPs were then required to recruit and review patients with asthma five times over a six month period. Collaborative practice was evaluated through a series of process measure. Attitudes toward collaboration/health care teams was evaluated using the Attitudes Towards Health Care Team Scale (ATHCTS).
Results. A total of 37 pharmacists, 13 general practitioners and 2 practice nurses recruited 312 patients with asthma. No significant difference was detected in ATHCTS between Groups 1, 2 and 3 over time. Of the 945 patient completed only 5% were seen by both a GP and pharmacist (10% in Group 1, 11% in Group 2 and 3% in Group 3). Of these visits 81%, 94% and 37% were entered on the electronic patient log by HCPs from Groups 1,2 and 3 respectively.
Discussion. Achieving collaboration in primary care remains a challenge, despite the method of training, the availability of a model of practice and clinical support. Future research should involve the Pharmacy Practice Incentives, which can provide a mechanism of remuneration within the current health care environment.
Perceived barriers to pharmaceutical services by clients with mobility, vision and hearing disabilities
Victor Chuang1, Pascale Ng Cheong Tin1, Richard Parsons1, Desiree Bui1. School of Pharmacy, Curtin Health Innovation Research Institute, Curtin University1, Perth, WA.

Introduction. Twenty per cent of the Australian population live with functional disabilities. Structural problems such as lack of wide doorways and ramps, communication difficulties and lack of provider knowledge about disability-related issues are barriers for people with disabilities to receive timely, high quality pharmaceutical services.

Aims. To investigate the perceived barriers of people with mobility, hearing and vision impairment in accessing pharmaceutical services and to determine whether these are different in non-disabled persons.

Methods. Self-administered surveys, available on paper and online, were collected from adults without a disability and from adults who have mobility, hearing or visual impairment from WA and other states in Australia. All participants took part in the study voluntarily; those with a disability were recruited through contacting various organisations and advertising the survey on disability-related internet websites. Data were analysed using the SPSS statistical software.

Results. 170 valid responses were identified for analysis (110 with disabilities and 60 non-disabled). Small writing on the label was the main barrier for the non-disabled (30%) and vision impaired (71.4%) groups. This was also a common barrier for the other two groups. The mobility disabled group (72.7%) had problems with a cluttered pharmacy, whereas the hearing disabled group (40.8%) felt uncomfortable with the way pharmacist communicated with them. Both the non-disabled and mobility disabled groups reported physical barriers such as small space and high counter level, while the vision and hearing disabled groups identified communication barriers such as the lack of suitable information formats as well as modes of interaction as major impediments. Only 76% of the whole group indicated that they were confident about their medicines when leaving the pharmacy.

Discussion. The study has identified areas for improvement in the provision of pharmacy service at primary care settings not only to clients with disabilities, but also the non-disabled clients.

ASCIA Anaphylaxis Training for Pharmacists
Rhonda M Clifford1, Sandra Salter1, Richard Loh2, Sandra Vale2, Jill Smith2. 1 Pharmacy, UWA, Nedlands, WA; 2 Australasian Society of Clinical Immunology and Allergy (ASCIA) (Introduced by Rhonda M Clifford)

Introduction. Maintaining knowledge in anaphylaxis management may save lives. The Australasian Society of Clinical Immunology and Allergy (ASCIA) provides lecture-based and e-training anaphylaxis courses for Australian health professionals, school and childcare staff.

Aim: To assess the effectiveness of ASCIA anaphylaxis lectures for pharmacists.

Methods. Approved ASCIA members presented the ASCIA lectures. Australian pharmacists and pharmacy students who attended lectures and agreed to participate were included. Effectiveness (gain and retention of knowledge) was measured using a twelve-item test administered pre, post, three and seven months after lecture completion. Mean pre and post scores were compared to determine knowledge gain. Mean scores pre and three, and pre and seven months after course completion were compared to determine retention. Frequencies were used to determine the pass rate for each test (scores $> 9/12$ (75%)).

Results. Pre and post-tests were completed by 152 pharmacists and 62 students, with 89 pharmacists (59%), and 45 students (73%) completing 3-month tests. Seventy-eight pharmacists (52%) and 34 students (55%) completed 7-month tests. Mean knowledge gain was 4.3 points ($p<0.001$). Mean retention at 3 and 7 months was 2.5 points ($p<0.001$) and 2.9 points ($p<0.001$) respectively, with retention at 7 months being significantly greater than at 3 months ($p<0.001$). Mean gain and retention was significantly greater for students compared to pharmacists (gain: 5.7 vs 3.7 points; retention: 3-months 3.8 vs 1.9; retention: 7-months 4.2 vs 2.4), all $p<0.001$. More pharmacists than students achieved a pass at every test: 97% vs 85%; 60% vs 53% and 80% vs 62% scored $> 9/12$ for post, 3 and 7-month tests respectively.

Discussion. ASCIA anaphylaxis lectures for pharmacists significantly increased short and long-term knowledge. Pharmacists consistently achieved higher pass rates than students, with the majority at seven months retaining sufficient knowledge to achieve a pass.
Risk factors for chlamydia: A survey of pharmacy-based emergency contraception consumers in Australia.
Rhonda Clifford¹, Sajni Gudka¹, Aline Bourdin¹, Kim Watkins¹, Atefeh Eshghabadi¹, Alan Everett¹. ¹Pharmacy Programme, UWA, Crawley, WA. (Introduced by Rhonda M Clifford, UWA, Crawley)

Introduction. In 2005, the Australian federal government stated that chlamydia screening programs should target the following: sexually active people aged 15-29 years, those that have experienced inconsistent use of barrier contraception, those with multiple sexual partners, and those with a history of a sexually transmitted infection.

Aims. To determine the prevalence of the above mentioned risk factors in pharmacy-based emergency contraception (EC) consumers; evaluate their pharmacy experience; and determine if they would be willing to accept a chlamydia test from the pharmacy in Western Australia.

Methods. A survey for women to complete after their EC consultation was developed from themes indentified in a literature search. 24 pharmacies participated in this study.

Results. From 113 surveys completed, 85% were 16-29 years of age and all (100%) women had inconsistent use of barrier contraception. 94% of the women had at least two, and 47% had at least 3 out of the 4 risk factors. 70% of women found pharmacy very easy/easy to access a pharmacy and felt very comfortable/comfortable discussing EC with the pharmacist. Most (72%) said they would accept a chlamydia test from a pharmacy.

Discussion. Women requesting EC from a community pharmacy are at high risk of chlamydia. Yet there is no mechanism by which pharmacists can request a chlamydia test in Australia. There is an urgent need to re-orientate health service so that pharmacists can offer women requesting EC a chlamydia test.

Off-label and unlicensed prescribing in a Western Australian paediatric population
Petra Czarniak¹, Jeff Hughes¹, Richard Parsons¹, Lewis Bint² & Bruce Sunderland¹. School of Pharmacy, Curtin Health Innovation Research Institute, Curtin Univ¹, Perth, WA. Princess Margaret Hospital², Perth, WA

Introduction. Unlicensed and off-label prescribing in paediatrics is a global phenomenon due to a lack of adequate registrations of paediatric drugs and formulations. Data on the extent of off-label and unlicensed prescribing in paediatrics in Australia is limited.

Aims. To evaluate the extent of off-label and unlicensed prescribing trends in a large paediatric hospital in Western Australia.

Methods. 1037 randomly-selected medication chart records from a single year (2008) from Princess Margaret Hospital for Children (PMH) were analysed for prescribing trends in paediatric emergency department admissions, outpatients and inpatients. Relevant patient data, prescribing details, diagnosis and adverse effects, were collected. Drugs were classified according to the ATC code. Standard statistical tests were applied.

Results. Most records (n=403; 39%) were from the Emergency Department; 37% as outpatients (n = 382) and 24% as inpatients (n = 253). A majority were males (58% in ED, 55% outpatients, 65% inpatients). There were 2660 drugs prescribed to 700 patients with inpatients administered significantly more drugs per person than emergency outpatients (p < 0.0001). Of the 253 inpatients, 154 males (79%) and 63 females (74%) received one or more off-label or unlicensed drug (p < 0.0001). The overall extent of off-label or unlicensed prescribing in all settings was 28%, with the greatest number administered to inpatients aged 2 to 5 years (mean 1.9). The most common ATC categories with off-label prescribing included the nervous system (44%), alimentary tract (20%) and anti-infectives (14%). Drugs commonly prescribed off-label included ondansetron, Painstop Day, salbutamol, oxycodone, paracetamol, midazolam, fentanyl, amoxycillin, flucloxacinil and ticarcillin with clavulanic acid.

Discussion. Off-label prescribing was common at PMH, with inpatients more likely to receive them than outpatients or emergency admissions. Although off-label prescribing is widespread, a paediatric formulary is essential to provide up-to-date and evidence based information on their uses.
**Medicines and their management among the older-aged living independently in leasehold retirement villages**

Sheila A Doggrell¹, Michelle Maugham¹, School of Biomedical Sciences, Queensland University of Technology¹, Brisbane, QLD

Introduction. Half of the older-aged, living in rental retirement villages, are nonadherent, or at risk of being nonadherent. They had a good knowledge of their illnesses for about half of their prescribed medicines, whereas those older-aged living in freehold rental retirement villages were much more adherent and had a better knowledge of their medicines/illnesses (Doggrell & Kairuz, 2012). Most of the older-aged that live in retirement villages live in leasehold villages. Unfortunately, initially we were unable to recruit enough participants from leasehold villages to assess their management of medicines.

Aim. The aim was to recruit participants from leasehold retirement villages, and compare their adherence and knowledge of medicines to those in rental and freehold retirement villages.

Method. Semi-structured interviews with the researchers rating their perception of the adherence, as described previously (Doggrell & Kairuz, 2012).

Results. The mean age of the participants at the leasehold retirement village was 83 years ±2 (n=22). Our perception was that 55% of the older-aged were fully adherent and unlikely to have problems in the next 6-12 months, whereas 41% were presently fully adherent but at risk of not being so in the future. The participants were taking 9.8±1 medicines each, with the commonest medicines being cardiovascular, followed by gastrointestinal and respiratory medicines. With regard to relating medicines to illnesses, the participants were good at this for 58% of medicines, and only had some or no knowledge of the medical use of their other medicines.

Discussion. The older-aged in leasehold villages were intermediary between the predominantly fully adherent in the freehold villages and the less adhering older-aged in the rental villages. Like the residents of the rental retirement village, many of the participants from the leasehold village did not have a good understanding of which illnesses their medicines were being prescribed for.


---

**The role of a medication incident reporting system in monitoring and signalling medication safety risks in primary care**

Khaled Eddie¹, Timothy F Chen¹, Andrew J McLachlan¹², William B Runciman³⁴, Romano A Fois¹, Fac of Pharm, Uni of Syd¹, NSW; Ctr for Educ & Res on Ageing, Concord RG Hosp², NSW; Sch of Psych, Soc Wk & Soc Pol, Uni of SA³, Adel, SA; Aust Pt Saf Fndn⁴, Adel, SA.

Introduction. One objective of the National Medicines Policy is to ensure timely and uninterrupted access to medicines through efficient distribution networks. Manufacturing problems can disrupt medicine supply and create quality and safety concerns for patients and health-care providers (HCPs). A medication incident (MI) reporting system may play surveillance and signalling roles by detecting the impact of and response to disruptions to medicine supply.

Aims. To identify an incident cluster for disruption to medicine supply from incident reporting data and reveal contributing factors and preventive strategies.

Method. Thirty community pharmacies in Sydney participated in a 28-month incident reporting study (QUMwatch) providing data on the nature of MIs in primary care. Data classification, management and analysis utilised AIMS® software. Process classifications and narrative text was searched to identify incidents associated with supply.

Results. 952 MIs were reported and 395 were analysed. MIs occurred during all stages of the medication use process. A cluster of spontaneous MI reports, related to disrupted supply for extended-release metformin products, signalled safety concerns including patient confusion, treatment interruption, ineffective communication from manufacturers and deficient processes and tools to advise and support HCPs and patients in selecting alternative therapies.

Discussion. A MI reporting system can identify risks, such as medication supply disruptions, and their underlying causes and generate strategies to prevent or moderate recurrence of emerging risks. A multidisciplinary national reporting system may facilitate large-scale trend analyses across a number of real-world situations to manage risks from medicines use.
Identification of medication errors amongst healthcare providers and academics in Denpasar Bali
Desak K Ernawati¹,², Jeff D Hughes¹, Ya Ping Lee¹. School of Pharmacy, Curtin Univ¹, Perth, WA; School of Medicine, Udayana Univ², Denpasar, Bali

Introduction. The Joint Commission on Accreditation of Healthcare Organizations in the United States has identified the prevention of adverse drug events, which include medication error, as a major health initiative. The ability to identify medication errors is crucial for healthcare practitioners in order to ensure the quality and safe use of medications.

Aims. To compare the ability to identify medication error amongst healthcare providers (physician, nurses and pharmacist) at Sanglah Hospital and medical, nursing and pharmacy academics at Udayana University in Denpasar, Bali.

Methods. The study utilises a 10 question survey questionnaire. Participants were recruited from 9 community pharmacies, over 3-6 weeks. Data analysis was performed using the Qualtrics Survey Software.

Results. A total of 47 completed surveys were returned to the research team. The characteristics of the menstrual cycles of the participants were comparable to the published literature, regarding length of menstrual cycle, duration of menstrual bleeding, and nature of PMS symptoms. Among the participants the most commonly used non-prescription products were the non-steroidal anti-inflammatory drugs (87%). Other products being used were EPO (13%) and vitex (4%). The greatest efficacy was reported from subjects who had been using EPO followed by ibuprofen and naproxen.

Discussion. The study identifies that women’s perception of the efficacy of these products does not correspond to currently available clinical data, in which vitex is the only product that has clinically proven efficacy and safety for PMS and EPO has no clinical data to support its efficacy. Future studies are required to examine the safe and effective use of non-prescription products in PMS.

The perceived efficacy of non-prescription medications used by women who experience PMS
Sarira Elden¹, Kwang Choon Yee¹ & Mary Madden¹. Pharmacy Discipline, SELS, EHSE, Charles Darwin University¹, Darwin, NT. (Introduced by R. Patel, Univ of Tasmania, Hobart, Tasmania).

Introduction. Premenstrual syndrome (PMS) is a multi-factorial condition which includes a wide range of symptoms and affects women of reproductive age. PMS is often self-diagnosed and treated by women. Various pharmaceutical preparations are available for the treatment of PMS, with varying degrees of clinical evidence. However, consumers’ choice is not always based on clinical evidence.

Aims. This study aims to examine the consumers’ perceived efficacy of non-prescription products marketed for the relief of PMS and period pain, which include vitex, evening primrose oil [EPO], ibuprofen, mefenamic acid, and naproxen.

Methods. The study utilises a 10 question survey questionnaire. Participants were recruited from 9 community pharmacies, over 3-6 weeks. Data analysis was performed using the Qualtrics Survey Software.

Results. A total of 47 completed surveys were returned to the research team. The characteristics of the menstrual cycles of the participants were comparable to the published literature, regarding length of menstrual cycle, duration of menstrual bleeding, and nature of PMS symptoms. Among the participants the most commonly used non-prescription products were the non-steroidal anti-inflammatory drugs (87%). Other products being used were EPO (13%) and vitex (4%). The greatest efficacy was reported from subjects who had been using EPO followed by ibuprofen and naproxen.

Discussion. The study identifies that women’s perception of the efficacy of these products does not correspond to currently available clinical data, in which vitex is the only product that has clinically proven efficacy and safety for PMS and EPO has no clinical data to support its efficacy. Future studies are required to examine the safe and effective use of non-prescription products in PMS.
A taste of your own medicine: prevalence of symptoms and self-medication in the community
James Green, Mudassir Anwar, Elitsa Hineva, Pauline Norris, Tom Elliott, Chanelle Kissick, Hannah Giles, Vanessa Ott, Amber McAuslan, Lauren Wylie, Josh Vereker-Bindon, Shannon Francois. School of Pharmacy, University of Otago, Dunedin, New Zealand

Introduction. Understanding the prevalence of symptoms and the responses people make to these symptoms, including self-medication, is key to encouraging optimal use of medicines. However, most research either engages with people who are sick enough to have sought advice from a healthcare professional, or asks people to try to recall past illness and treatment. These will however miss a substantial proportion of self-medication, where no advice is sought, or the symptoms and treatment are not recalled, due to their minor or passing nature.

Aims. To gain insight into the prevalence of symptom experience and self-medication in a community-living population, using a prospective cohort design.

Methods. Random sampling from the Dunedin White Pages was used to recruit participants aged 18-65. After filling out an initial questionnaire, participants were contacted on daily basis via email or text message for 30 days, asking if they had experienced symptoms in the last 24 hours. If symptoms were experienced, participants filled out a longer online questionnaire about their symptoms, whether they had self-medicated, and if they had consulted a healthcare professional.

Results. 154 participants were recruited, with 152 completing 30 days. Over the 30 day period 83.5% reported at least one symptom, with headache (60.6%) being the most prevalent. Using a medicine already in the house was the most common response on any symptomatic day (40.8%) with only a small proportion of participants choosing to seek professional medical help. Despite participants rating pharmacists as the best choice for treating minor symptoms, doctors were more commonly consulted than pharmacists.

Discussion. Symptoms were experienced frequently by participants, many of which would be unlikely to be recalled in a retrospective design. Self-medication was the most common response, with evidence of non-optimal medicines use, suggesting the need for strategies to improve self-medication and engagement with pharmacists.
The impact of the rescheduling of combination analgesics containing codeine on the practice of pharmacists
Ainslie M Hamer1, M Joy Spark 1, Penelope J Wood 1, Emily Roberts 2, School of Pharmacy and Applied Science, La Trobe University1, Bendigo, VIC1; Emily Roberts Amcal Pharmacy2, Bendigo, VIC

Introduction. On the 1st May 2010 combination analgesics containing codeine (CACC) changed from Schedule 2 to Schedule 3 meaning that the involvement of a pharmacist is now involved in all sales.
Aims. To explore how the rescheduling of CACC has impacted on the practice of community pharmacists.

Methods. A descriptive qualitative design was used, with data collected via face-to-face semi-structured interviews that were recorded and transcribed verbatim. The data was analysed thematically via open, axial and selective coding.

Results. From the eleven pharmacists interviewed, it was found that pharmacists monitor the supply of CACC by recoding sales and will intervene when they feel that the medication is being overused. Pharmacists perceived there to be a number of challenges surrounding the provision of CACC. These relate to inconsistent procedures between pharmacies, being unaware of CACC supply from other pharmacies and how to assist dependent people.

Discussion. The extent to which pharmacists record sales varies with some recording all sales, whilst others selectively record for certain patients or products. This different approach can lead to patients feeling singled out or cases of overuse not being detected. Pharmacists also felt that inconsistent procedures at different pharmacies are leading to customer misunderstandings. Respondents expressed the desire for a national monitoring system so that they would be aware of patients purchasing CACC from other pharmacies, which is not currently possible because their records are limited to their own pharmacy. Strategies used by pharmacists when they detect overuse of CACC are educating patients about side effects, recommending alternative treatments, refusing sales and referring to the doctor. However, respondents lacked confidence to raise the issue of suspected misuse and codeine dependence and were unsure of where to send dependent people for help. Investigation into more effective ways of identifying and intervening in codeine dependence is required.

The effect of weak English skills on academic performance, and the effectiveness of English language screening and remedial help for pharmacy students
James A Green1. School of Pharmacy, University of Otago1, Dunedin, New Zealand

Introduction. English language proficiency is required for registration as a pharmacist in Australasia. Thus, educational institutions often screen to detect pharmacy students with weak English. The impact of weaker language skills, and the effectiveness of screening, is difficult to determine if students are selected on the basis of English language proficiency or a proxy (such as an interview). However, while pharmacy students at Otago are screened for English language prior to entry, they are selected solely based on grade average.
Aims. To examine the effect of weak English on performance in pharmacy education, and also the effectiveness of English language screening and remedial help.

Methods. A retrospective cohort study, comprising all students entering the programme directly from a common health science first year (HSFY) in 2007-2009. Relationships were examined between results from an HSFY screening test, subsequent screening tests in the pharmacy programme, and their academic grades and progress.

Results. Poor performance on English screening tests had a lasting impact on academic performance. Failing the HSFY and second year screening tests both predicted lower grades (Betas>-4.1, ps<.01), a greater likelihood of failing one or more papers (Odds ratio 2.9, p<.01), and a greater likelihood of having to repeat a year of the pharmacy programme (Odds ratio 3.0, p<.05), controlling for academic performance at entry. Almost all students who failed the HSFY test also failed a second screening test a year later, despite having successfully passed a semester long remedial course in between the tests.

Discussion. Difficulties with English have a pronounced effect on students’ performance in pharmacy education. English screening tests appear to be effective in identifying students with poorer English skills, but it may be difficult for students to improve their language skills while studying full time. This presents an ongoing challenge to pharmacy educators.
Concomitant use of alcohol and sedative-hypnotics in middle and older aged people: a systematic review

Jenni Ilomäki¹, Tapio Paljarvi², Maarit Korhonen³, Hannes Enlund⁴, Christopher P Alderman⁵, Jussi Kauhanen⁶, Simon Bell¹; Sansom Institute, School of Pharm and Med Sci, Univ of South Australia¹, Adelaide, SA; Dept of Public Health, Univ of Helsinki², Helsinki, Finland; Dept of Pharmacology, Drug Development and Therapeutics, Univ of Turku³, Turku, Finland; Finnish Medicines Agency⁴, Kuopio, Finland; Pharmacy Dept, Repatriation General Hospital⁵, Daw Park, SA; Faculty of Health Sciences, Univ of Eastern Finland⁶, Kuopio, Finland

Introduction. Interactions between alcohol and sedative-hypnotics may result in adverse events. Patterns of alcohol drinking and sedative-hypnotic drug use differ between countries.

Aim. To conduct a systematic review on the prevalence of concomitant alcohol and sedative-hypnotic use among middle-aged and older persons.

Methods. MEDLINE, EMBASE and PsycINFO (January 1990-present) were searched using Medical Subject Headings and keywords. Population-based studies reporting the quantity of alcohol drinking, prevalence of sedative-hypnotic use, and in which the mean age of participants was ≥40 years were included in the review.

Results. Five population-based studies conducted in North America, ten in Europe and one in Australia were included in the review. Up to 88% of men and 79% of women who used sedative-hypnotics also consumed alcohol. Up to 28% of those who consumed alcohol were concomitant users of sedative-hypnotics. Middle-aged people consumed higher quantities of alcohol and exhibited more risky drinking patterns, including binge drinking and heavy drinking, than older persons. In contrast, sedative-hypnotic use was more prevalent among older than middle-aged persons.

Discussion. Our review identified a higher prevalence of alcohol consumption among middle-aged than older persons. Middle-aged persons may experience harm from alcohol/sedative-hypnotic drug interactions due to risky drinking behavior. Older persons have a higher prevalence of sedative-hypnotic use and may be more susceptible to addictive central nervous system effects than younger persons due to physiologic changes in psychotropic drug and alcohol metabolism. Clinicians should consider patients’ alcohol consumption patterns before prescribing sedative-hypnotic drugs.

Commercial influences on community pharmacist recommendations – impact of the Extended and Accelerated Price Disclosure

Lillian Huang¹, Greg Kyle¹², Taso Raptis¹, Lisa Nissen¹
School of Pharmacy, University of Queensland¹, QLD, Discipline of Pharmacy, Canberra University², ACT

Introduction. Community pharmacy faces an inherent conflict between fulfilling professional responsibilities and maintaining a profitable business. We have previously shown that generic substitution by pharmacists was influenced by commercial factors including higher profitability. However, fulfillment of legal and professional requirements with these substitutions was not fully met. The first Main Disclosure Cycle of the Extended and Accelerated Price Disclosure (EAPD) occurred on 1 April 2012 and resulted in a 10-73% price reduction across a range of prescription medicines.

Aims. To examine if the pattern of generic substitution of prescription medicines is affected by the EAPD reduction in margin.

Methods. Ten community pharmacies around Brisbane were recruited to provide a range of business types and demographic settings. Observational studies were carried out after 1 April 2012 over six three-hour session and all prescription transactions were recorded. These results were analyzed to determine generic substitution rates and concordance with professional requirements, and also compared with our historical data which were collected before EAPD.

Results. It appears that the rate of generic substitution of prescription medications was increased across all study pharmacies. However, there appears to be an increase in the number of transactions that were not handled to meet the professional and legal requirements. Further data collection and analysis are underway.

Discussion. The increase in generic substitution appears to be a response to boost profitability after EAPD. This should in turn drive generic substitution and reduce government cost - an aim of EAPD. The decline in professional standards is of concern as increased substitution will require more explanation to prevent medication misadventure and provide QUM improvements to accompany the financial benefits of EAPD.
Timing of the Drug Administration in Clinical Practice in Australia
Gagandeep Kaur1. Faculty of Pharmacy, University of Sydney, Sydney, NSW.

Introduction. Timing of drug administration is important for health care professional and pharmacists. The concept of chronotherapy is an emerging field in healthcare, which deals with finding the optimal time of drug administration. This review scopes the evidence of chronotherapy in current clinical practice.

Aims. To find and evaluate the evidence of chronotherapy in top 20 (by volume) Pharmaceutical Benefit Scheme (PBS) drugs in Australia and to identify how well this information is considered in clinical practice though Monthly Index of Medical Specialties (MIMS).

Methods. The search was conducted in MEDLINE and IPA using the keywords “Drug Chronotherapy”, “Drug administration Schedule”, “Administration Time Dependent effects”, “Circadian Rhythms”, “Chronopharmacology”, “Chronopharmacokinetics”, “Chronopharmacodynamics”, “Morning and Evening”, “Morning and Bedtime”, “Morning and Night time” and their combination which was later filtered with top 20 PBS drugs sold in Australia. Articles were limited to English language, humans, year (1990-April 2012) and Clinical Trials.

Results. Our search revealed a total of 770 articles, of which 12 articles were selected for review. The evidence for chronotherapy that was tested in these 12 studies were Atorvastatin (n=2), Rosuvastatin (n=1), Simvastatin (n=3), Perindopril (n=1), Pantoprazole (n=1), Irbesartan (n=1), Atenolol (n=1), Rabeprazole (n=1) and Ramipril (n=1). The most common study design utilized in these studies was the Randomised Control Trial (RCT) (n=11). The timing of drug administration is defined for Simvastatin (evening) and Perindopril (morning) in MIMS.

Discussion. The study presents the scope of chronotherapy in clinical practice. Our analysis revealed three categories of drugs. (1) Chronotherapeutic drugs used in clinical practice, (2) The potential chronotherapeutic drugs not used in clinical practice and (3) The drugs that has chronotherapeutic potential but not researched. There is a need to bring awareness of chronotherapeutic drugs in clinical practice for health care professionals.
**Current tapering recommendations for discontinuing psychotropic medications: a systematic review.**
Greg J Kyle, Stephanie Salvage. Discipline of Pharmacy, University of Canberra, Canberra, ACT.

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 quantitative 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.

**Creating a smoke free University of Canberra.**
Greg J Kyle, Louise S Deeks. Discipline of Pharmacy, University of Canberra, Canberra, ACT.

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.
Development of Health Professional relationships in an Interprofessional Learning workshop.
Sofia Mavritsakis¹, Erica Sainsbury¹, Biljana Cvetkovski¹, Sinthia Bosnic-Anticevich¹, Faculty of Pharmacy, University of Sydney, Sydney, NSW.

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.

"I just have to get off my arse" Barriers to medication adherence in young adults with chronic disease.
Cobie B McQueen¹, Virginia A Dickson-Swift², Christina M Dennis¹. School of Pharmacy and Applied Science, La Trobe University¹, Bendigo VICTORIA; La Trobe Rural Health School, La Trobe University², Bendigo VICTORIA. (introduced by M. Joy Spark La Trobe University, Bendigo VICTORIA).

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.
Utilization of actigraphy to assess undiagnosed sleep disturbances among healthy adults in home-based settings
Zaswiza M Noor, Alesha Smith, Simon Smith, Lisa Nissen. School of Pharmacy, University of Queensland, Woolloongabba, QLD; Institute for Health and Biomedical Innovation and Centre of Accident Research and Road Safety, Queensland University of Technology, Kelvin Grove, QLD.

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

Thi-My-Uyen Nguyen1, Adam La Caze1, Neil Cottrell1, School of Pharmacy, Pharmacy Australia Centre of Excellence - University of Queensland1, Brisbane, QLD

Introduction. Improving medication adherence can reduce the risk of adverse health outcomes and healthcare 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.
Community pharmacists’ awareness of secondary prevention of cardiovascular disease: a preliminary study
Hanni P Puspitasari1,2, Parisa Aslani1, Ines Krass1. Faculty of Pharmacy, Univ of Sydney1, Sydney, NSW; Faculty of Pharmacy, Airlangga Univ2, Surabaya, Indonesia

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.

Career perspectives of final year Australian pharmacy students
Grace H. Shen, Romano A. Fois, Bandana Saini, Faculty of Pharmacy, Univ of Sydney, Sydney, NSW

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.

The use of herbal medicines in lactation among breastfeeding women in Western Australia: A population-based survey
Tin Fei Sim1, Jill Sherriff2, Lisa B.G.Tee1, School of Pharmacy, Curtin University1, Perth, Western Australia; School of Public Health, Curtin University2, Perth, Western Australia.

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 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.
Medicines prices to patients in Australia
Loc Thai, Agnes Vitry. Quality Use of Medicines and Pharmacy Research Centre, University of South Australia, Adelaide, SOUTH AUSTRALIA

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.

Accessibility of compounded progesterone products
M. Joy Spark, Jon Willis, Graeme Byrne, Teresa Iacono. School of Pharmacy and Applied Science, LaTrobe University, Bendigo, VIC; Aboriginal & Torres Strait Islander Studies Unit, University of Queensland, Brisbane, QLD; School of Mathematics & Statistics, LaTrobe University, Bendigo, VIC; School of Rural Health, LaTrobe University, Bendigo, VIC.

Introduction. There are few proprietary progesterone (P₄) products on the market in Australia, so women who use P₄ have to obtain their products from compounding pharmacies.

Aims. To identify components of accessibility to compounded P₄ products for Australian women.

Methods. A cross-sectional quantitative study was used to survey women about their experience of P₄. Participating pharmacies, across all Australian states, included a questionnaire with P₄ 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 P₄ treatment. Some groups of women were less likely to be concerned about other treatments and naturalness than others: younger women (≤50 years); if their doctor’s influence to start P₄ treatment had been large; if initiating P₄ treatment had not been influenced by other treatments not working well enough; and women whose prescribing doctor had been seen prior to commencing P₄. 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 a large influence. A university education enabled women to overcome the impediments of rurality and socioeconomic disadvantage.

Discussion. Women who have accessed P₄ 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.
Usage of heart failure medications in frail and robust older inpatients

Kristina J. Waddell1,2,3,4, Danijela Gnjidic1,2,3,5, Peter R. Carroll3, Slade T. Matthews3 & Sarah N. Hilmer2,3,4. Faculty of Pharmacy, Univ of Sydney1, Sydney, NSW; Dept of Clin Pharmacol and Aged Care, Royal North Shore Hosp 2, St Leonards, NSW; Sydney Medical School, Univ of Sydney3, Sydney, NSW; Kolling Institute, Royal North Shore Hosp4, St Leonards, NSW; Centre for Education and Research on Ageing, Concord Hosp5, Concord, NSW.

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; 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.
Retrospective review of initial management of febrile neutropenia at Flinders Medical Centre: Time to first dose of antibiotic therapy and risk stratification.
Diane Watson¹, Ganessan Kichenadasse², Richard Marotti¹, Vaughn Eaton¹. Dept of Pharmacy¹, Flinders Medical Centre, Adelaide, SA; Dept of Medical Oncology/Clinical Pharmacology², Flinders Medical Centre, Adelaide, SA. (Introduced by Ganessan Kichenadasse, Flinders Medical Centre, Adelaide, SA).

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 x 10⁹ 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.


A longitudinal study of constipation and laxative use in a community-dwelling elderly population
Barry L.Werth¹, Kylie A.Williams² & Lisa G.Pont¹. Sydney Nursing School, Univ of Sydney¹, Sydney, NSW; School of Pharmacy, Univ of Technology Sydney², Sydney, NSW.

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% to 14%). 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.
**Investigation of benzodiazepine utilisation in older people admitted to hospital**

Jackson Crawn, Juanita Westbury, Gregory Peterson. Unit for Medication Outcomes, Research and Education, School of Pharmacy, University of Tasmania, Hobart, TASMANIA.

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

Barbara C Wimmer1,2, Alexander B Mullen2,1School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SOUTH AUSTRALIA; 2Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, SCOTLAND.

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.

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

Elida Zairina1, Kay Stewart1, Michael J Abramson2,3, Johnson George1. Centre for Medicine Use and Safety, Monash University, Parkville, VICTORIA1; Dept of Epidemiology and Preventive Medicine, Monash University2, The Alfred Hospital, Melbourne, VICTORIA3.

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.

Elucidating drug release mechanisms of biomacromolecule-containing lipid particles during lipolysis
Philip Christophersen¹, Hanne M. Nielsen¹, Mingshi Yang¹, Anette Müllertz¹,² and Huiling Mu¹. ¹Department of Pharmacy, University of Copenhagen. ²Bioneer: FARMA, Department of Pharmacy, University of Copenhagen

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 mechanisms 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. CaCl₂ 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.
UV imaging and flow through Raman spectroscopy: information-rich tools for characterising the dissolution behaviour of furosemide

Sarah Gordon¹, Line Hagner Nielsen¹, Kaisa Naelapää¹, Jukka Rantanen¹, Jesper Østergaard¹, Anette Müllertz¹,². Dept of Pharm, Univ of Copenhagen, Copenhagen, DK; ²Bioneer: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) 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.
Addition of hydroxypropyl methylcellulose to furosemide increases physical stability of the amorphous form of furosemide

Line Hagner Nielsen¹, Thomas Rades¹, Anette Müllertz¹,². ¹Bioneer:FARMA, ¹Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark; ²Preformulation, H. Lundbeck A/S, Valby, Denmark

Introduction. Converting poorly soluble drugs to an amorphous form improves dissolution rate and solubility can be obtained

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 fur osemide formulations 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 μ-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/cm²/min for the amorphous salt and 1.3±0.1 mg/cm²/min and 0.45±0.07 mg/cm²/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.
Comparison of two extraction methods prior to chromatographic determination of metabolic fragments of beta-endorphin within inflamed rat tissue
Naghmeh Hajarol Asvadi¹, Michael Morgan¹, Amitha Hewavitharana¹, Paul N. Shaw¹, Peter J. Cabot¹, School of Pharmacy, The University of Queensland, Brisbane, Queensland¹.

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.

Polarized light microscopy as a method for analyzing API precipitation in simulated gastric media
Linda G. Jensen¹, Jukka Rantanen¹, Thomas Rades¹, Bertil Abrahamsson² and Anette Müllertz¹,³. ¹Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark. ²AstraZeneca R&D Mölndal, Pepparedsleden 1, SE-431 83 Mölndal, Sweden.
³Bioneer:FARMA, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

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 0.5μL was placed on a microscope slide. 19.5μL 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 1875μm x 1405μm (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±123μm, with 75% being shorter than 350μm, 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.
Stability of risperidone in a novel polyol-based low-aqueous in situ gelling emulsion
Tanya Jones1,2, Ross Norris2, Alison Haywood1. School of Pharmacy, Griffith University1, Gold Coast, QLD; Australian Centre for Paediatric Pharmacokinetics, Mater Pharmacy Services2, South Brisbane, QLD.

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.

Nicotine retention as the major determinant of percutaneous absorption of nicotine
Rina Kuswahyuning1,2, Peng Li1, Michael S Roberts1,3. School of Pharm and Med Sci, Univ of South Australia1, Adelaide, SA; Fac of Pharm, Univ of Gadjah Mada2, Yogyakarta, IND; School of Med, Univ of Queensland3, Woolloongabba, QLD.

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

Yady J Manrique¹, Kathryn J Steadman¹, Faiza Islam¹, Lisa M Nissen¹, Julie Cichero¹ and Jason Stokes².

School of Pharmacy¹, School of Chemical Engineering². The University of Queensland, Brisbane, QLD.

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.
Posters:

A mechanistic based approach for enhancing buccal mucoadhesion of chitosan

Emil Meng-Lund¹,Christian M. Westergaard¹, Camilla Sander¹, Peter Madelung¹ and Jette Jacobsen¹. Department of Pharmacy, University of Copenhagen, Denmark. ²Drug Delivery, Disposition and Dynamics, Monash University, Melbourne, Victoria.

Introduction. Mucoadhesive formulations for drug administration into the oral cavity have attracted increased attention in 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 discs 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.

Validation of Microtitre plate assay for Haemophilus influenzae spp.Biofilm

Najla Obaid¹, Stephen Tristram², Glenn Jacobson¹. (School of Pharmacy, University of Tasmania¹, Hobart, TAS; School of Human life Sciences, University of Tasmania, Launceston, TAS²).

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% CO₂, the cell density adjusted and broth inoculated into 4 wells of a 24 well microtitre plate then 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.

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

Zong-Quan Ou1, David Schmierer1, Clare Strachan1,2, Thomas Rades1,3, and Arlene McDowell1. New Zealand’s National School of Pharmacy, University of Otago1, Dunedin, New Zealand; Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki2, Helsinki, Finland; Department of Pharmacy, University of Copenhagen3, Copenhagen, Denmark.

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 in 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-1-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.


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

Pernille B. Pedersen1, Peter Vilmann2, Daniel Bar-Shalom1, Stefania G. Baldursdottir1, and Anette Müllertz1. Dept of Pharmacy, Uniy of Copenhagen1, Copenhagen, DK. Copenhagen Uniy Hosp Herlev2, Herlev, DK

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

Washington Y. Sanchez1, Vânia R. Leite-Silva1, Marina Le Lamer1,3, David C. Liu1,4, Washington H. Sanchez2, Isabel Morrow5,6, Darrel Martin5, Tarl W. Prow4, Jeffrey E. Grice1 and Michael S. Roberts1,7.

University of Queensland, 1Therapeutics Research Centre, Woolloongabba, QLD; Universidade Federal de São Paulo, Instituto de Ciências Ambientais Químicas e Farmacêuticas2, Diadema SP, Brasil; Claude Bernard University, Faculty of Pharmacy3, Lyon, France; University of Queensland, Dermatology Research Centre4, Woolloongabba, QLD; University of Queensland, ARC Centre of Excellence for Functional Nanomaterials5, Australian Institute for Bioengineering and Nanotechnology, Brisbane, QLD; The University of Queensland, Australian Microscopy and Microanalysis Research Facility6, Brisbane, QLD; University of South Australia, 7School of Pharmacy and Medical Science, Adelaide, SA (introduced by Washington Y. Sanchez, Therapeutics Research Centre, University of Queensland, Woolloongabba, QLD).

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, r² = 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.


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

Washington Y. Sanchez1, Vânia R. Leite-Silva1,2, Marina Le Lamer1,3, David C. Liu1,4, Washington H. Sanchez2, Isabel Morrow5,6, Darrel Martin5, Tarl W. Prow4, Jeffrey E. Grice3 and Michael S. Roberts1,7.

University of Queensland, 1Therapeutics Research Centre, Woolloongabba, QLD; Universidade Federal de São Paulo, Instituto de Ciências Ambientais Químicas e Farmacêuticas2, Diadema SP, Brasil; Claude Bernard University, Faculty of Pharmacy3, Lyon, France; University of Queensland, Dermatology Research Centre4, Woolloongabba, QLD; University of Queensland, ARC Centre of Excellence for Functional Nanomaterials5, Australian Institute for Bioengineering and Nanotechnology, Brisbane, QLD; The University of Queensland, Australian Microscopy and Microanalysis Research Facility6, Brisbane, QLD; University of South Australia, 7School 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.
**611**

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


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)NH₂) 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)NH₂, 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.


**612**

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

Mohamed Sharkasi¹, David E. Hibbs¹, W. Bret Church¹, Stuart Cordwell², Nathan Hare², Paul W. Groundwater¹. Faculty of Pharmacy¹, and School of Molecular Bioscience², Univ. of Sydney, Sydney, NSW 2006, Australia (introduced by Andrew McLachlan, Univ. of Sydney).

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

Bradley S Simpson¹, David J Claudie², Nick M Smith¹, Ross A McKinnon³ and Susan J Semple. Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia¹, Adelaide, SA; Chuulangun Aboriginal Corporation², Cape York Peninsula, QLD; Flinders Centre for Innovation in Cancer, Flinders University³, Bedford Park, SA.

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.

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

Daniel T Barratt¹, Ene-choo Tan², Alex T Sia³, Janet K Coller¹ & Andrew A Somogyi¹. Disc Pharmacology, Univ of Adelaide¹, Adelaide, SA; KK Research Centre and Dept of Women’s Anaesthesia, KK Women’s & Children’s Hosp², Singapore.

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.
The pharmacogenomics knowledge, education, practice and attitudes of hospital pharmacists in Adelaide, South Australia
Mafalda M Dias1, Helena M Ward2, Michael J Sorich3, Ross A McKinnon3. School of Pharmacy & Medical Sciences, UniSA1, Adelaide, SA; School of Medicine, Flinders University2, Adelaide, SA; Flinders Centre for Innovation in Cancer, Flinders University3, Adelaide, SA.

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.


A pharmacogenomic study investigating outcomes in advanced non-small cell lung cancer patients receiving paclitaxel and carboplatin therapy with a focus on ethnic differences
Benjamin D W Harris1, Andrew J McLachlan2, Stephen J Clarke3, Kellie Charles1. Discipline of Pharmacology, Univ of Sydney1, Camperdown, NSW; Centre for Education and Research on Ageing, Concord Repatriation General Hospital (CRGH)2, Concord, NSW; Department of Medical Oncology, Royal North Shore Hospital3, St Leonards, NSW.

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 (Ȥ2 = 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

Zaipul I Md Dom1,2, Janet K Coller2, Andrew A Somogyi2, Benedetta C Sallustio1,2. Dept of Clin Pharmacol, Queen Elizabeth Hosp1, Woodville, SA; Discipline of Pharmacol, School of Medical Sciences, Univ of Adelaide2, Adelaide, SA.

Introduction. Multidrug resistance protein 2 (MRP2), a membrane efflux transporter expressed on hepatocyte canalicular membranes, is encoded by \textit{ABCC2}, for which a number of single nucleotide polymorphisms (SNPs) (\textit{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 \textit{ABCC2} polymorphisms may affect MPA pharmacokinetics. There are conflicting reports regarding the role of MRP2 on MPA pharmacokinetics, however the role of \textit{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 \textit{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. \textit{ABCC2} genotyping (\textit{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 disequilibrium was observed between the \textit{C-24T} and \textit{C3972T} SNPs (\textit{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) \textit{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.

Laechelt et al (2011) Pharmacogenomics J 11:25-34
Characterisation of spontaneous activity in the human prostate gland
Basu Chakrabarty1, Mark Frydenberg2, Nathan Lawrentschuk1, Gail Risbridger3, Betty Exintaris1. Drug Discovery Biol, Monash Institute of Pharmaceutical Sciences1, Melbourne, VIC; Prostate and Breast Cancer Res Group, Dept of Anat and Dev Biol, Monash Univ2, Melbourne, VIC; Dept of Surg and Ludwig Institute for Cancer Res, Univ of Melbourne, Austin Health3, Melbourne, VIC; Dept of Surg, Monash Univ4, Melbourne, VIC.

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 Ca²⁺ 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.


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

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 μmol/L – 1 mmol/L) (n= 6; P < 0.001) and o-methylene ATP (3 mmol/L – 10 μmol/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 o-methylene ATP induced contraction implies the extract engenders an effect either at P2X7-purinoceptors or along the intracellular pathway activated by ATP.

Distribution of 5-HT receptors and interacting proteins in human colonic tissue layers
Helen R Irving1, Kenneth A Chinkwo1,2, Nor Yaakob1, Navinisha Chetty1, Paul V Desmond3, Ian M Coupar1. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University 1, Parkville VIC; School of Biomedical Sciences, Charles Sturt University 2, Wagga Wagga NSW; Department of Gastroenterology, St. Vincent’s Hospital, University of Melbourne 3, Fitzroy VIC.

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-HT3 and 5-HT4 receptors. 5-HT3 receptors interact with RIC3, whereas 5-HT4 and 5-HT7 receptors interact with GRKs and Lin 7 homologues. Aims. To examine the distribution of 5-HT 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-HT4 and 5-HT7 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-HT3A receptor expression was detected throughout the colon while 5HT3E 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.

624

Mitomycin C alters urothelial ATP, acetylcholine and PGE2 release in vitro
Sung-Hyun Kang1, Russ Chess-Williams1 & Catherine McDermott1. Bond University1, 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 PGE2 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 PGE2) 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 PGE2 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 ≥ 0.2 mg/mL. 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 PGE2 from RT4 cells was also observed immediately following treatment.

Discussion. The findings indicate that release of Ach, ATP and PGE2 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.


625

Effects of acrolein, a metabolite of Cyclophosphamide and Ifosfamide, on cultured human urothelial cells
Kylie A Mills1, Catherine McDermott1, Russ Chess-Williams1. Faculty of Health Sciences & Medicine, Bond University1, 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 (0.01–100μM) or acrolein (0.01–100μM) 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 100μM. Basal and stimulated acetylcholine release was not altered by acrolein treatment, however at a concentration of 100μM acrolein caused a 5-fold increase in basal and 2.5-fold increase in stimulated ATP release (Figure 1).

Discussion. Acrolein (100μM) alters urothelial cell viability and ROS production, while the parent drugs (100μM) do not. Stretch of the urothelium during bladder filling is known to stimulate the release of ATP which acts on low threshold δ 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.

Figure 1: Effect of 24-hour 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.
P-glycoprotein expression level in treatment - resistant Helicobacter pylori patients
Marhanis S Omar, Andrew Crowe & Jeffery Hughes. School of Pharmacy, Curtin University, Perth, WA.

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-glycoprotein 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.

Nerve-evoked and phasic contractions of the rat bladder: effects of low testosterone and treatment with the selective androgen receptor modulator trenbolone
Donna J Sellers1, Roselyn Rose’Meyer2, Joss du Toit2, Daniel Donner2, Fac of Health Sci & Med, Bond Univ1, Gold Coast, QLD; School of Med Sci, Griffith Univ2, Gold Coast, QLD.

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 (10µM) 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 (1µM) 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 (100µM).

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.

Koritsiadis et al. (2008) BJU Int 101:1542-46
Complexity in 5-HT3 receptors: An optimized expression system for electrophysiology studies
Nor S Yaakob, Betty Exintaris, Helen R Irving. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University VIC

Introduction. 5-HT3 receptors fall in the family of ligand-gated cation channels. Their presence in both central and peripheral nervous systems has been implicated in a range of clinical disorders such as irritable bowel syndrome, chemotherapy-induced nausea, schizophrenia and autism. 5-HT3 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-HT3 receptor subunits in mammalian cells. This is to ultimately examine whether different 5-HT3 receptor compositions and clinically-relevant SNPs in recombinant 5-HT3 receptors produce different electrophysiological activities.

Methods. cDNA encoding 5-HT3C, 5-HT3E and 5-HT3A subunits plus GFP were all cloned into one multicistronic 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

Low prevalence of Helicobacter pylori infection among patients with ulcerative colitis in Ukraine
Tetyana Ternuschak¹, Ivan Chopey¹, Ksenia Chopey¹, Andrey Bratasuk¹ Vasilii Ploskina¹
¹Chair of Therapy and Family Medicine, Uzhhorod National University, Uzhhorod, Ukraine.

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 mucosa.

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.

Changes in vimentin distribution accompany acrolein toxicity in epithelial lung cells: Association with protein adduct distribution
Kimberley E Burton, Philip C Burcham & Peter J Henry, School of Medicine & Pharmacology, University of Western Australia, Perth, WA.

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 μM) 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.


Acute acrolein exposure produces molecular, morphologic and functional changes in airway epithelium as investigated in a novel ex vivo mouse tracheal perfusion system
Esther Y Cheah, Philip C Burcham, Tracy S Mann & Peter J Henry. School of Medicine & Pharmacology, Univ of Western Australia, Perth, WA.

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 secretagogue ATP (100nM, 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 (200nM for 30min) did not affect responses to the epithelial-dependent relaxant substance P or the smooth muscle relaxant PGE2 (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.
Expression of TLRs and GFAP in the rat intestine following chemotherapy for cancer and relationship to gut toxicity and central pain behaviour
Janet K Coller1, Joanne Bowen2, Mark R Hutchinson2, Rachel Gibson3. Pharmacology, University of Adelaide1; Physiology, University of Adelaide2; Anatomy & Pathology, University of Adelaide3; Adelaide, SA.

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 jejunum 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 TLR4 and 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.

Safety and toxicity profile of fenugreek
Song H Eow1, Andrew Crowe1, Lisa BG Tee1. School of Pharmacy1, Curtin University, Perth, WA.

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 (50μg/mL, 100μg/mL, and 250μg/mL), with cell viability down to 10% at 100μg/mL. However, Rh123 efflux was unchanged with a 6 fold efflux ratio for both normal Caco-2 monolayers and those incubated with 50μg/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.
Structure toxicity studies of drugs implicated in immune mediated idiosyncratic hepatotoxicity

Samuel Ho¹, David Hibbs¹, Associate Professor Timothy Chen¹, Romano Fois¹ and Professor Andrew McLachlan¹². Faculty of Pharmacy, Univ. of Sydney¹, Concord Hospital²

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 eosinophilia (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 attributed 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 hepatotoxicity 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 yielded specificities and sensitivities 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)

Christopher I. Johnston1,2, Margaret A. O’Leary3, Simon G.A. Brown4, Bart J. Currie5, Randall Greenberg6, Michael Taylor7, Chris Barnes8, Julian White9, Geoffrey K. Isbister2,3 for the ASP investigators. School of Medicine Sydney, University of Notre Dame Australia1, Darlinghurst, NSW; NSW Poisons Information Centre, Sydney Children’s Hospital Network 2, NSW; Dept of Clinical Toxicology and Pharmacology, Calvary Mater Newcastle Discipline of Clinical Pharmacology, University of Newcastle3, Newcastle, NSW; Centre for Clinical Research in Emergency Medicine, Western Australian Institute for Medical Research, Royal Perth Hospital and University of Western Australia4, WA; Menzies School of Health Research and Northern Territory Clinical School, Royal Darwin Hospital5, Darwin, NT; Emergency Dept, Dubbo Base Hospital6, Dubbo, NSW; Emergency Dept, Albury Base Hospital7, NSW; Emergency Dept. Bundaberg Hospital8, Bundaberg, QLD; Dept of Toxinology, Women’s and Children’s Hospital9, Adelaide, SA.

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

Alice E Kane, Aniko Huizer-Pajkos, Victoria C Cogger, David G Le Couteur & Sarah N Hilmer. Syd. Med. School, Univ of Sydney, Sydney, NSW; Dept of Clin Pharmacol & Aged Care, Royal North Shore Hosp, St Leonards, NSW; Kolling Inst., Royal North Shore Hosp, St Leonards, NSW; ANZAC Research Inst., Concord Hosp, Concord, NSW.

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 (50μM 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 (50μM) 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 (50μM) 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 50μM 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.


---

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


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.

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.

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

Helen E Ritchie1, Deena H Ababneh2, Diana J Oakes1, Carl A Power3, William S Webster2. Discipline of Biomedical Science, University of Sydney1, Sydney, NSW. Discipline of Anatomy and Histology, University of Sydney2, Sydney, NSW. Biological Resources Imaging Laboratory, University of NSW3, Sydney, NSW. (Introduced by Diana J Oakes, University of Sydney, Sydney, NSW).

Introduction. Dofetilide is an If blocker (If = 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.

Webster et al (1996) Teratology 53(3) 168-175

Chronic low dose exposure to STX inhibits neurite outgrowth

Katie O’Neill1, Ian F Musgrave1, Andrew Humpage2. Dept of Pharmacol, School of Med Sc, Univ of Adelaide, Adelaide, SA1; Australian Water Quality Centre, Adelaide, SA2.

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 μg/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 (0.25-3μg/L) for 7 days with toxin and growth medium replaced on day 4. Concentrations were chosen based on the 3μg/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 than 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.

**POSTER ABSTRACTS**

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

Danielle L Perichon¹, Andis Graudins¹ ², Dept of Pharmacology¹, Monash University, Clayton, Vic; Southern Clinical School², Monash Medical Centre, Clayton, VIC

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≤0.02); amitriptyline area-under-curve was also significantly greater (p≤0.01) 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.

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

Chin Lin Wong¹ ², Ai-Leen Lam¹ ², Bruce D Wyse¹ ², Maree T Smith¹ ². Centre for Integrated Preclinical Drug Development, Univ of Queensland¹, Brisbane, QLD; School of Pharmacy, Univ of Queensland², Brisbane, QLD.

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, monocyctic 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.