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Role of inflammation in Neuropsychiatric disorders

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Introduction: The concept of an "immune privileged" CNS has been redefined to suggest that the CNS is a site of selective and modified immune reactivity [1]. The findings presented provide mechanistic and clinical evidence to suggest that phenotypes of neuropsychiatric disorders such as cognitive and affective dysfunction and neurodegeneration are influenced by pathological functional and morphological changes of the CNS induced by impaired immune functions, and by neuroinflammation in particular. Under immune challenged conditions such as Encephalitis and Multiple Sclerosis, inflammation in the CNS results in trafficking of B and T cells to the site of injury leading to impaired pathophysiological mechanisms relevant to neuropsychiatric disorders.

Aim: This presentation will demonstrate the current understanding on the role of inflammation in neuropsychiatric disorders.

Results: The activation of humoral- and cell-mediated immunity is met with an upregulation of various inflammatory proteins such as cytokines and chemokines. It has been demonstrated that these immune cells traffic to various regions in the CNS including the hippocampus, a region regarded as essential for memory and learning. A chronic upregulation of such inflammatory proteins may result in dysfunction of key cytokine-mediated molecular mechanisms and neuronal–glial interactions that subserve synaptic plasticity and learning and memory processes in the brain. During pathological conditions microglial interact with neurons, possibly via P2x7 receptors, to induce a neuroinflammatory response characterized by an up-regulation of cytokines, such as, IL-1, IL-6 and TNF, which can then alter the function of cytokines in synaptic plasticity [2]. In addition, chronic inflammatory conditions in the CNS may lead to pathological morphological changes such as grey matter hypointensities and associated iron deposition in basal ganglia as seen in diseases like Multiple Sclerosis [3] associated with cognitive impairment and neuropsychiatric symptoms.

Discussion: The above described mechanisms provide insight into shared pathologogical mechanisms of distinctly different neurological and psychiatric disorders such as depression, psychosis and cognitive decline / dementia.

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B Cell Subtypes in Atherosclerosis. Therapeutic Implications

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Atherosclerosis is a chronic inflammatory disorder responsible for the majority of deaths due to myocardial infarctions and strokes. Atherosclerotic lesions that develop in the vessel wall of medium/large arteries are the result of complex interactions between accumulated LDL-cholesterol, endothelial and smooth muscle cells and cells of the innate and adaptive immune system. Multiple immune cells accumulate in atherosclerotic lesions including macrophages and dendritic cells, NK and NKT cells, CD4+ and CD8+ T cells and B cells. Early studies indicated that B cells are protective against atherosclerosis by producing low affinity IgM antibodies against oxidised LDL (low density lipoprotein). However, recent advances in B cell immunobiology indicate multiple subtypes of B cells suggesting a more complex role in the pathogenesis of atherosclerosis. Using an anti-CD20 B cell depleting antibody, we reinvestigated the role of B cells in atherosclerosis using fat fed ApoE-/- mice. Using such mAbs we demonstrated that B cell depletion decreases development and progression of murine atherosclerosis. Adoptive transfer approaches demonstrated that the B2 B cell subtype was proatherogenic. By targeting the B2 B cell survival factor BAFF we demonstrated their important role in atherosclerotic lesion inflammation and development/progression of atherosclerosis. Unlike B2 B cells, B1a B cells produce natural IgM antibodies as well as interleukin-10. In contrast to B2 B cells, deletion of peritoneal and splenic B1a B cells aggravates atherosclerosis. These effects were accompanied by marked reductions in anti-oxidised LDL IgM antibodies, lesion apoptotic cell numbers and necrotic core development. Therapeutically targeting these cells with anti-TIM (RMT1-10) mAb attenuates atherosclerosis.

Therapeutic targeting of the innate immune complement system in neurodegenerative disease

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Introduction. There is increasing evidence that neuroinflammation drives the progression of neurodegenerative disease. This study explored the role of the potent inflammatory complement activation fragment, C5a, in two mouse models of motor neuron disease, and Parkinson's disease.

Methods. SOD1^{G93A} transgenic mice were used as a model of motor neuron disease, and striatal injections of 6-hydroxydopamine (6-OHDA) used to induce Parkinson's disease in mice. Expression of complement and C5a receptors (C5aR) were examined in the central nervous system of animals through qPCR, Western blotting, and immunohistochemistry, at defined ages through disease progression. Separate mice were also treated with the selective cyclic peptide C5a receptor (C5aR) antagonist, PMX205 (hydrocinnamate-[OPdChaWR]), in the drinking water.

Wild-type spinal cord (P170) — Red = Complement activation

SOD1^{G93A} spinal cord (P170) — Red = Complement activation

Results. Complement was found to dramatically activated during disease progression in both models of neurodegenerative disease (see Figure). This correlated with increases in C5aR expression, predominantly on activated microglia surrounding regions of neuronal death. Oral drinking water treatment with PMX205 was first demonstrated to cross the blood brain barrier, confirming the ability of this drug to block central nervous system C5aRs. SOD1^{G93A} motor neuron disease mice treated with PMX205 had reduced motor deficits and extended survival compared to untreated mice. Similarly, 6-OHDA Parkinson's disease mice showed markedly reduced behavioural and motor deficits compared with untreated mice. In both models, PMX205 treatment was found to ameliorate microgliosis in affected brain regions.

Discussion. Our findings demonstrate that complement activation, C5aR upregulation, and ultimately C5aR signalling are key events in these neurodegenerative models. Reducing C5a-mediated microglial neuroinflammation using specific pharmacological inhibitors could be an important therapeutic strategy to treat a wide variety of neurodegenerative diseases.
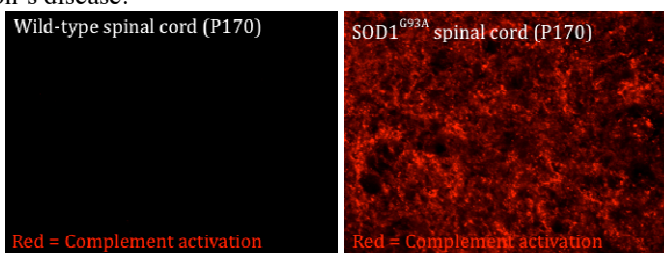
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Mineralocorticoid receptors, not just salt and water

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Introduction. Mineralocorticoid receptor (MR) activation in the presence of high salt promotes vascular and cardiac inflammation, remodeling and fibrosis. A recent study showed mice in which the macrophage MR is selectively deleted, are protected from hypertension and cardiac inflammation and fibrosis without apparent change in macrophage recruitment.

Aims. Establishing and characterising the cellular mechanisms of MR signalling in cardiac inflammation, fibrosis and high blood pressure.

Methods. To dissect the pathological role of the MR in specific cell types of the cardiovascular system in the development of cardiovascular disease, we have generated transgenic mice containing tissue selective deletion of the MR and investigated responses to well characterised cardiovascular disease models.

Results. Comparison of responses in transgenic and wild type mice to models of cardiovascular disease have revealed novel roles for the MR in several different cell types of the heart and vasculature. We have shown that MR activation in monocytes/macrophages is required for cardiac tissue remodelling in DOC/salt and L-NAME/salt models of disease. While recruitment of macrophages to the heart remains unchanged the proinflammatory and profibrotic phenotype of the macrophages is altered suggesting a central role for macrophage MR in these disease models. More recent studies in cardiomyocyte MR null mice show that the cardiomyocyte MR plays an independent role in the tissue remodelling process in addition to an important role in chemoattractant signalling for inflammatory cell types.

Discussion. Macrophage recruitment and activation via MR-dependent mechanisms play a significant role in the regulation of systolic blood pressure and cardiac inflammation and fibrosis. The MR has many other important and distinct roles in many of the cell types in the heart and vasculature in the development of heart disease. Understanding the down stream signalling pathways of the MR in the cell types critical for the onset and progression of disease may allow for the development of targeted therapies for heart failure that avoid the side affects associated with current MR blockers.

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The importance of basic drug metabolism research to the pharmaceutical industry: Success in the past 30 years and needs for the future

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Introduction. Cytochrome P450 (P450, CYP) enzyme are involved in > 75% of enzymatic drug metabolism reactions.

Aims and Methods. Fifty years after the discovery of P450, there has been a remarkable contribution of the science in the field of drug discovery, development, and practice. Some classic examples of success are with ethynylestradiol, terfenadine, and warfarin.

Results and Discussion. In a sense, P450 is a mature field. We now have structures of at least 20 human P450s, and every pharmaceutical company has P450 screens in its armamentarium. Where do we go from here? In addition to several questions about more basic science (to be discussed), four major areas have been identified. First, there is room for more practical applications of pharmacogenetics in clinical practice. Why has progress not been faster? Second, what are the "orphan" P450s (those without defined functions) doing and how important are they? Third, how do human P450s influence cancer risk? Finally (fourth), do P450s have roles in chromic disease other than cancer and is intervention possible?
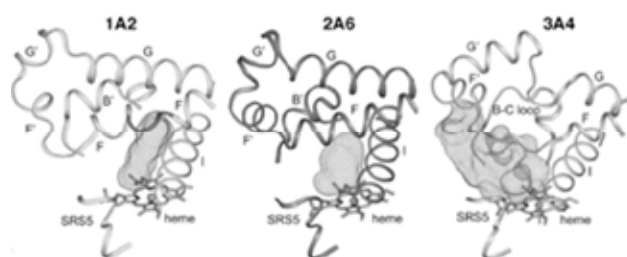
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Innovative approaches for the assessment of pharmacokinetics and drug-drug interactions
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Pharmacokinetic variability is one of the greatest challenges in drug therapy due to the huge differences of drug concentration at the site of action. Among many factors CYP3A plays a very prominent and significant role in pharmacokinetic variability and also in drug-drug interactions. Because so many drugs are metabolised by this enzyme the current CYP3A activity would very valuable in terms of drug dosing to individualise drug therapy. Midazolam can successfully used as a probe drug to determine CYP3A activity via the the partial metabolic clearance of the CYP3A pathway. Using standard doses of midazolam produce pharmacological effects especially when administered under inhibitory conditions. A 1000-fold lower midazolam dose does not impose any risk for a pharmacological effect, it can safely be administered to every patient. In combination with a limited sampling methodology using 4 samples only, a frequent CYP3A activity measurement can be performed. CYP3A activity is altered by drugs which inhibit or induce CYP3A, the extent of alteration depends on the dose (exposition) of the perpetrator but not on the dose of the victim. The outcome of a combination of an inhibitor and an inducer depends on the concentration and the potency of the inhibitor wheather there is net inhibition or net induction. Administration of the same dose of a CYP3A substrate results in large variability of drug exposure (> 10-fold) which in consequence translates to large variability of drug effects. Determination of CYP3A activity and individually calculated doses can reduce this variability dramatically. Targeted dosing of CYP3A substrates might be possible with the knowledge of the CYP3A activity, taking emerging information on the impact of other contributing factors like drug transporters or other enzymes.

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Substrate selectivities and structure-function relationships of human cytochrome P450 enzymes
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Enzymes of the cytochrome P450 (CYP) superfamily are responsible for the metabolism of a myriad of compounds that include drugs, non-drug xenobiotics (e.g. environmental chemicals), and endogenous compounds. The individual P450 enzymes exhibit characteristic substrate and inhibitor selectivities, and enzyme activities are variably affected by age, disease states, epigenetic regulation, ethnicity, genetic polymorphism, and hormonal factors. Not surprisingly, wide interindividual variability in clearance (and hence response) is often a feature of drugs metabolised by P450 enzymes. However, knowledge of the P450 enzyme(s) responsible for the metabolism of a compound ('reaction phenotyping') allows prediction, at least at the qualitative level, of factors likely to alter clearance in patients. In vitro approaches for the reaction phenotyping of P450 substrates have been developed in recent years, although the reliability of reaction phenotyping is critically dependent on experimental conditions. Moreover, the structural features of P450 proteins that confer substrate and inhibitor selectivity, including the contribution of individual amino acids to ligand binding, are becoming increasingly understood from site-directed mutagenesis, x-ray crystallography, and protein homology modelling. While x-ray crystallographic data provide valuable insights into P450 substrate/inhibitor binding domains, the structures elucidated are static and data are limited in terms of the thermodynamics of binding and movements of individual amino acids. Molecular dynamics simulations provide a means to model the thermodynamics and flexibility of proteins, and conformational changes can be studied in detail at the atomic level over time. It will be demonstrated how molecular dynamics simulations have allowed us to differentiate binding modes and key amino acids for acidic ('typical') and basic ('atypical') substrates of CYP2C9.

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Influenza drugs – more than an effective drug is needed for success
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The development of the world's first specific anti-influenza drug Relenza relied on cross-discipline collaborative efforts between CSIRO, academia and industry. Relenza is administered by a dry powder inhalation, which delivers the drug directly to the upper respiratory tract, the site of virus replication. The second drug on the market, Tamiflu is taken orally. Despite its efficacy, and the large numbers of inhaled medications used globally, the psychological acceptance of Relenza as an inhaled drug has been poor. Since getting a drug to market is estimated to cost around $1 billion, there is less interest now by industry in investing in second/third generation drugs. This is despite resistance emerging to primarily Tamiflu, and hence the need for expanding the drug repertoire stockpiled for pandemic preparedness. However, preliminary evaluation is still being carried out between academia and industry, but government funding is needed to fully develop these new drugs. Since influenza remains both a pandemic and a potential bioterrorist threat, there is some US government support through both BARDA and the NIH/NIAID for the development of second and third generation inhibitors. The Biomedical Advanced Research and Development Authority (BARDA), within the Office of the Assistant Secretary for Preparedness and Response in the U.S. Department of Health and Human Services, can assist in the development of vaccines, diagnostics and therapies for public health medical emergencies. In addition to direct grant funding, the NIAID can also provide preclinical evaluation of drugs through their contract laboratories. You do not need to hold an NIH grant. However, the preference appears to be primarily for an orally available anti-influenza drug, despite efficacy of the inhaled drugs. No matter how safe and effective the drug is, there needs to be a return on investment by the big pharma, and importantly psychological acceptance of the mode of administration.

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The Discovery of Novel Anticancer Agents: The Industry-Academic Interface
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Introduction. The development of novel targeted therapies for the treatment of cancer is a significant focus of research in both industry and academia worldwide. Targeted therapies have a greater likelihood to possess improved efficacy and reduced toxicities compared to conventional cytotoxic agents. As our understanding of the fundamental drivers of cancer development and growth improves, new molecular pathways are being identified that represent ideal targets for therapeutic intervention.
Aims. This presentation will outline some of the exciting programs underway in anticancer drug discovery at the Walter and Eliza Hall Institute (WEHI). Our rationale for undertaking these particular drug discovery projects and our engagement with industry will also be presented.
Methods. Our drug discovery programs are based on both structurally-guided 'rational design' medicinal chemistry programs, as well as phenotypic screening approaches to identify new drug-like molecules and chemical probes for study in models of disease and ultimately clinical development.
Results. A summary of WEHI's successful collaboration with Abbvie-Genentech will be presented along with ongoing programs directed towards kinase and epigenetic targets.
Discussion. Drug discovery is a complex and multidisciplinary undertaking ideally suited to be conducted in a research institute such as WEHI where we have immediate access to world-class science, infrastructure, and domain experts and clinicians. Nonetheless, the focus on scientific publications and requirement for grant funding does present specific challenges for drug discovery scientists working in academia. Successful navigation through these issues by partnership and collaboration with the pharma and biotech industry can allow for the discovery and development of truly novel first-in-class drugs.

Experiences in collaborative Drug Discovery and Development
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Introduction. Effective collaborations do not arise *de novo*. They require conscious and deliberate planning and rigorous execution. The journey can be long and arduous, but if properly structured and managed, it can be rewarding and lucrative. The process starts with the assembly of a well led, motivated team focused on a competency or product that the prospective collaborator is expected to need and presenting it in a manner that is liable to attract interest. Subsequent licensing involves raising awareness of the opportunity, identifying and engaging with potential partners, surviving the due diligence process and reaching agreement on contracts and project governance. These processes are important to not only lay the legal framework but also the cultural and practical environment in which the collaborators will subsequently interact. Every relationship and project is different but success factors can be identified that lead to productive collaborations.

Discussion. Understanding the current and future market for your project is key to determining the competitive position and the potential value to a partner. Excellent science is not enough; disciplined and adaptive project management systems must be in place to guide projects even at an early stage. In our experience, a clearly defined target product profile provides the beacon and helps develop a common understanding across disciplines to guide programs. Successful projects rely on trust, mutual respect and the alignment of business and R&D team cultures towards shared goals. This requires effective alliance management structures and open communication at all levels of the partnership. Biota projects have received funding from pharma companies, governments and charitable trusts. Our experiences of different collaborations and the changing landscape of biotech and pharma partnering will be discussed.

Drug development in a University setting - factors for success
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Every drug development project is a unique combination of different origins, rates of progress, funding sources, collaborations and outcomes. A review of successful projects in the Auckland Cancer Society Research Centre (those resulting in a drug taken to clinical trial) suggest a number of important drivers. The most important is close multi-disciplinary collaboration, initially within the University project group and critically, at the appropriate time, an equally close collaboration with a commercial partner (be that a startup company, a biotech or a big pharma). This will be illustrated with reference to four disparate projects on prodrugs for cancer therapy, a new drug for persistent tuberculosis and a drug for transplant therapy.

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Functional analysis of novel bitter ligands in rodent and human cardiac tissue *ex vivo*

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Introduction. G protein-coupled receptors (GPCRs) are key mediators in cardiovascular physiology, yet the frontline therapies for heart disease target only a small fraction of the cardiac GPCR repertoire. Moreover, there is emerging evidence that taste GPCRs have specific functions beyond the oral cavity. Our recent description of taste GPCRs in rodent and human heart (Foster *et al.*, 2013) provides a platform for further study on the function of these previously unappreciated cardiac GPCRs.

Aims. To investigate the effects of bitter ligands on cardiac function *ex vivo* in rodent and human heart tissue.

Methods. Right atrial appendage tissues were dissected from patients undergoing coronary artery bypass grafts and/or aortic valve replacement at The Prince Charles Hospital. Intact trabeculae were mounted onto tissue electrode blocks and changes in contractility were recorded in response to putative taste GPCR ligands. Hearts were isolated from 8 week old male C57BL/6 mice, and perfused in Langendorff mode. Coronary flow, aortic pressure and left ventricular pressure were recorded during infusion of putative taste GPCR ligands identified in a compound screen.

Results. In human right atrial strips, addition of several bitter compounds (1 mmol/L final) elicited a striking loss in contractile force, (e.g. denatonium benzoate 89±5%, n=5; control 12±5% reduction of contractile force; n=5; P<0.0001). In mouse hearts, all *Tas2r* ligands tested exhibited concentration-dependent effects on cardiac function. The biphasic change of aortic pressure with sodium benzoate and the sodium thiocyanate-mediated decrease in systolic pressure were both abrogated in the presence of Gα$_i$ (pertussis toxin) and Gβγ (gallein) inhibitors.

Conclusion. This study represents the first demonstration of profound bitter ligand-induced, G protein-dependent effects on cardiac function in rodent and human tissues. Future work will focus on the delineation of specific G protein and receptor-dependent responses of these ligands.

Foster SR, *et al.* (2013). *PLoS One* 8(5): e64579.

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Membrane-dependent control of cardiac function, ischaemic tolerance and opioidergic protection

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Introduction. Opioid-mediated sustained ligand-activated preconditioning (SLP) provides potent protection and signalling appears distinct to conventional preconditioning. Various studies support essential roles of caveolins and caveolae, particularly caveolin-3, in cardiac tolerance to ischaemia-reperfusion (IR). Reductions in caveolar density and caveolins may contribute to age/disease-related impairment of IR tolerance. Methyl-β-cyclodextrin (MβCD) is an agent commonly used to deplete membrane cholesterol and disrupt caveolae.

Aims. To assess effects of cholesterol depletion on intrinsic cardiac tolerance and protection mediated via conventional and novel modalities. Additionally, we sought to determine the dependence of SLP-mediated protection upon caveolin-3.

Methods. Langendorff-perfused hearts from young male C57/Bl6 mice were subjected to no treatment or 25 min pre-treatment with various concentrations of MβCD (2 μM – 1 mM) prior to 25 min ischaemia/45 min reperfusion. Langendorff-perfused caveolin-3 (cav-3) knock-out and overexpressor hearts were also exposed to IR ± SLP. Cholesterol content was assessed using Amplex Red Cholesterol Assay kit.

Results. Pre-treatment with MβCD significantly reduced sarcolemmal cholesterol content from >10-30% in a concentration-dependent manner (20 μM – 1mM), and induced significant pre- and post-ischaemic contractile dysfunction compared to untreated hearts. Exogenous post-ischaemic cardioprotection with acute morphine (10 μM) was abolished with >20 μM MβCD, however SLP remained efficacious until 200 μM MβCD. Interrogation of caveolin-3 role in the SLP phenotype revealed that SLP improved post-ischaemic recovery in cav-3 knockout and protection was additive in cav-3 overexpressor hearts.

Discussion. This study highlights the importance of membrane cholesterol upon both intrinsic tolerance and response to ischaemia-reperfusion. Moreover, cholesterol depletion attenuates the ability to precondition the heart against injury via conventional and novel modalities. Novel SLP is further delineated from conventional preconditioning stimuli, as this phenotype appears independent of cav-3 protein and caveolae.

NOX2 oxidase deficiency promotes plaque stability in advanced atherosclerosis

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Introduction. Atherosclerosis is a chronic disease, yet many of its complications (e.g. myocardial infarction, ischemic stroke) are a result of an acute, physical disruption to a lesion leading to thrombosis. Consequently, the stabilisation of atherosclerotic plaques represents an important preventive therapeutic strategy. Enhanced oxidative stress, in particular, the superoxide ($^\cdot O_2^-$) generating enzyme NOX2 oxidase, has been implicated in early atherogenesis (Drummond et al, 2011), yet little is known about its effects upon plaque stability.
Aim. To determine if limiting $^\cdot O_2^-$ generation, via genetic deletion of NOX2 oxidase, is protective in atherosclerosis.
Methods. Plaque stability was determined through histological analysis of lipid, collagen, α-smooth muscle cell actin and macrophage content in innominate arteries from 26 week-old male C57Bl6/J (WT), apolipoprotein E-deficient (ApoE$^{-/-}$) and ApoE$^{-/-}$/Nox2$^{-/y}$ mice maintained on a high fat diet (21% fat, 0.15% cholesterol) for 21 weeks. Plasma cholesterol levels and aortic superoxide $^\cdot O_2^-$ production (chemiluminescence) and nitric oxide (NO) bioavailability (contraction to L-NAME) were also assessed.
Results. ApoE$^{-/-}$ mice displayed elevated total plasma cholesterol (~6.9-fold, P<0.001), increased aortic $^\cdot O_2^-$ generation (~1.7-fold, P<0.05), reduced endogenous NO bioavailability (~25%, P<0.05) and atherosclerotic lesion development (n=7-9) when compared to WT mice. The absence of NOX2 in ApoE$^{-/-}$/Nox2$^{-/y}$ mice resulted in a ~70% reduction in aortic $^\cdot O_2^-$ production (P<0.001, n = 7-9) with no change in endogenous NO bioavailability (n = 7-9). Although plasma cholesterol and lesion area were similar in ApoE$^{-/-}$/Nox2$^{-/y}$ versus ApoE$^{-/-}$ mice, NOX2 deletion induced changes in plaque composition that promoted plaque stability. Thus, collagen and α-actin accumulation were increased by ~2.4-fold (P<0.01) and ~1.9-fold (P<0.05), respectively and lipid deposition was reduced ~40% (P<0.01). Lesional macrophage content was unchanged (n = 9).
Discussion. NOX2 deletion is associated with reduced vascular $^\cdot O_2^-$ production and improved plaque stability. Thus, targeting NOX2 oxidase may protect against cardiovascular complications associated with atherosclerosis.
Drummond G et al (2011) Nat Rev Drug Discov 10:453-471

A novel model using weight change to describe the disease progression of type 2 diabetes

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Introduction. A previously developed semi-mechanistic model (de Winter et al, 2006) described changes in fasting serum insulin (FSI), fasting plasma glucose (FPG) and glycosylated haemoglobin (HbA1c) in type 2 diabetic (T2DM) patients, by modelling the underlying process of insulin sensitivity and beta-cell function. Weight, in particular change in weight (DWT), is associated with diabetes onset and management. Weight loss is correlated with lowering glucose (Henry et al, 1986), which has been attributed to regained insulin sensitivity.
Aims. To mechanistically incorporate current physiological knowledge of the relationship between weight and FPG/FSI in a population model to describe the effect of weight change on disease progression of T2DM.
Methods. Non-linear mixed effects modelling using NONMEM 7.2 (Beal et al, 2009) was performed on placebo arm data from 181 newly diagnosed obese T2DM patients (mean baseline weight = 104kg). The subjects were counselled on diet and exercise (D&E) throughout the study. At start of study, 7 weeks after screening, patients received placebo for 60 weeks. D&E was modelled as an inhibitory effect on weight input, and DWT was allowed to affect insulin sensitivity (IS), which drives the FPG-FSI homeostasis.
Results. D&E had an overall weight loss effect on the patients (mean DWT = -4.1kg at end of study), which resulted in a decrease in FPG (8.0 to 7.5 mmol/L) and HbA1c (6.8 to 6.5%) peaking at around 90 days. The estimated baseline values for beta-cell function and IS were 61.3% and 24.8% of normal, respectively. The patients regained an estimated 27% of their innate IS at the end of the study.
Discussion. Weight change was successfully implemented to the semi-mechanistic disease progression model for T2DM. As T2DM is intricately linked with obesity, further application of this updated model could prove useful in understanding the disease.

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Unravelling the mechanism of TGF-β-induced epithelial glucocorticoid resistance through Next-Generation Sequencing (RNA-seq)

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Introduction. Glucocorticoid (GC) resistance limits the successful treatment of chronic inflammatory diseases. We have identified TGF-β as a novel inducer of GC insensitivity in bronchial epithelial cells. However, the molecular mechanism of this resistance is unknown. Extensive investigations into known TGF-β signalling pathways have revealed this resistance is not dependent on SMAD4, and cannot be prevented by inhibiting known non-canonical pathways. A non-hypothesis driven approach is therefore required.

Aim. To use Next Generation Sequencing (RNA-seq) to facilitate efforts to reveal the mechanism of TGF-β-induced GC resistance.

Methods. RNA-seq was performed on RNA extracted from BEAS-2B cells treated 24h with 40pM TGF-β then 4h with 30nM dexamethasone (Dex) using an Illumina HiSeq™ 2000 sequencing platform. Changes from control of more than 2.5 fold were analysed as significant changes and a subset of the observed expression changes were confirmed by RT-qPCR.

Results. Dex up-regulated 108 genes in total. Six of these that were up-regulated by TGF-β alone were removed to prevent confounding analyses. Sixty-six genes were only up-regulated by Dex in the absence of TGF-β, and 36 genes were still up-regulated by Dex in the presence of TGF-β. *In silico* analysis using the miRror suite (http://www.proto.cs.huji.ac.il/mirror/index.php) has identified three families of micro RNA (miRNA) that differentially regulate these gene sets.

Discussion. RNA-seq analysis identified 2 sets of genes up-regulated by GCs in bronchial epithelial cells, one of which remains inducible and the other which is rendered insensitive to GC activation in the presence of TGF-β. Understanding the effects of dysregulated microRNAs on GC action may reveal novel therapeutic targets to treat GC resistance.

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Altered protein expression with secretory pathway calcium ATPase 1 (SPCA1) silencing in MDA-MB-231 breast cancer cells

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Introduction. The secretory pathway calcium ATPase 1 (SPCA1) is a Ca^{2+} pump localized to the Golgi apparatus, a major site of protein trafficking and processing. SPCA1 is upregulated in some basal-like breast cancers, a molecular subtype of breast cancer that infers a poor prognosis. Recent evidence suggests that SPCA1 may be involved in regulating the expression of proteins important in cancer progression.

Aims. To identify protein(s) with altered expression due to SPCA1 silencing in MDA-MB-231 basal breast cancer cells using 2D-DIGE and explore the mechanisms and functional consequences of such altered protein expression.

Methods. MDA-MB-231 cells were seeded into 6-well plates (75,000 cells/well) and treated with SPCA1 (treated) or non-targeting (control) siRNA. Protein was isolated 72 h post siRNA treatment and silencing was confirmed by real time RT-PCR and immunoblotting. 2D-DIGE and MS/MS were used to identify proteins with differential expression with SPCA1 silencing. Altered protein expression of target proteins were validated by immunoblotting.

Results. Heat shock protein 60 (HSP60) was identified as a protein target potentially sensitive to SPCA1 silencing using 2D-DIGE. Immunoblotting validated HSP60 protein expression was downregulated by 81±2% (n=3, P<0.05) upon SPCA1 silencing in MDA-MB-231 cells. Real time RT-PCR revealed that SPCA1 silencing and pharmacological inhibition of NFκβ activity reduced HSP60 mRNA levels by 60±7% and 43±4% respectively (n=3, P<0.05). SPCA1 silencing resulted in altered sensitivity to staurosporine-induced cell death but did not alter sensitivity to heat-shock-induced cell death.

Discussion. 2D-DIGE is a suitable approach to identify proteins sensitive to SPCA1 silencing in MDA-MB-231 cells. SPCA1 silencing leads to the transcriptional and translational downregulation of HSP60, a chaperone protein involved in cell death pathways. Although further studies are required to fully characterize the functional consequences HSP60 downregulation, this work suggests SPCA1 is a complex regulator of proteins important in cancer pathways.

Targeting type-1 interferon signalling is neuroprotective in the MPTP mouse model of Parkinson's disease.

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Introduction: Neuroinflammation has recently been implicated in contributing to Parkinson's disease (PD) pathology. Type-1 interferons (IFNs) are known to initiate/regulate the neuroinflammatory cascade; however their involvement in PD is unclear. Previously, we identified increased levels of type-1 IFNs in post mortem human PD brains. In addition, we demonstrated that the neurotoxin rotenone up-regulates type-1 IFN production and signalling *in vitro*, with IFN-α receptor-1 knockout (IFNAR1$^{-/-}$) neurons protected against rotenone-induced toxicity.

Aims: This study investigated the role of type-1 IFNs in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) mouse model of PD.

Methods: Wildtype and IFNAR1$^{-/-}$ mice (n=10) were administered MPTP (4x15mg/kg, 2h intervals) before brains were harvested at 1, 3 and 21 days post MPTP for RNA, protein and histological analysis.

Results: QPCR analysis confirmed IFNAR1$^{-/-}$ mice demonstrated significantly reduced levels of IFNα and IFNβ, compared to wildtype mice (n=6, p<0.01). IFNAR1$^{-/-}$ mice also exhibited decreased type-1 IFN signalling with reduced STAT-3 phosphorylation identified by western blot. Significantly, IFNAR1$^{-/-}$ mice displayed a reduced pro-inflammatory phenotype, with decreased levels of IL-1β, TNF-α and IL-6 as measured by QPCR and ELISA (n=6, p<0.05). In addition, reduced microglial activation (Iba-1^{+}) and astrogliosis (GFAP) was identified by immunohistochemistry in the substantia nigra (SN) of IFNAR1$^{-/-}$ mice 3 days post-MPTP. Overall IFNAR1$^{-/-}$ mice had increased survival of SN neurons compared to wildtype (3907±64 versus 3079±70, n=10, p<0.001), identified by tyrosine hydroxylase quantification, 21 days post MPTP.

Discussion: These results have identified a role for type-I IFNs as critical mediators of the detrimental neuroinflammatory response in MPTP mouse model of PD. Targeting type-1 IFN signalling may provide a novel therapy to reduce neuroinflammation, and hence limit neuronal cell death in PD.

Effect of ageing and paracetamol on the intrinsic death pathway in Fischer 344 rat livers

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Introduction. Drug induced liver injury results in apoptotis that is regulated by mitochondria *via* the intrinsic death pathway. In young mice, following an acute toxic dose of paracetamol, pro-apoptotic (BID, BAX, BAK) and anti-apoptotic (Bcl-2, Bcl-Xl) proteins translocate into the mitochondrial membrane where their pivotal balance determines apoptosis. The effect of ageing on this toxicodynamic process is unknown.

Aims. To characterise the degree of hepatotoxicity and expression of the intrinsic death associated proteins after administration of a toxic dose of paracetamol to young and old male Fischer 344 rats.

Methods. Young adult (6 ± 1 months) and old (26 ± 2 months) male 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 using ketamine (75mg/kg)/xylazine(10mg/kg) ip. Serum ALT and liver histology indicated the degree of hepatotoxicity. Samples of perfused liver were snap frozen for subsequent immunoblot analysis of BAX, BAK, BID, Bcl-Xl and Bcl-2 expression in the mitochondria and cytosol, VDAC- 1 in the mitochondria and Caspase-3 in cytosol.

Results. Serum ALT was elevated significantly in paracetamol treated young rats. Paracetamol did not alter expression of the intrinsic death pathway in young or old rats. In saline treated animals, cytosolic expression of pro-apoptotic BAX, BAK, BID and anti-apoptotic Bcl-Xl were decreased in old rats compared to young *(p<0.05)*. With old age mitochondrial pro-apoptotic BAK and BID expression significantly decreased *(p<0.05)*, while anti-apoptotic Bcl-2 trended to decrease, and BAX, Bcl-Xl and VDAC-1 did not change. Caspase-3 activation and mitochondrial BAX : Bcl-2 ratio (apoptosis markers) increased in old age *(p<0.05)*.

Discussion. These results suggest that the intrinsic death pathway is not activated by paracetamol and pro-apoptotic changes occur in this pathway with ageing in male Fischer 344 rats.

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Building bridges across the silos: Developing evidence-based guidance for intravenous paracetamol use in the paediatric population

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Introduction: The approved Australian Product Information (PI) for intravenous (IV) paracetamol was changed in 2009, halving the dose in infants weighing <10kg to 7.5 mg/kg/dose. Additional concerns followed industry and regulatory correspondence in 2012.

Aims: To review the evidence for the changed dose and provide evidence-based guidance on appropriate and safe use of paracetamol in the paediatric population.

Methods: A literature review was undertaken to identify the extent of and underlying reasons for paracetamol toxicity. Published research evidence, unpublished data, and up-to-date paediatric prescribing information was reviewed using a systematic process. Input was obtained from a multidisciplinary group of health professionals with a range of therapeutics, medicines evaluation and clinical expertise (paediatric and adult). A draft guidance document was circulated for consultation with external organisations and clinicians. Feedback was reviewed and discussed to refine the final guidance.

Results: No reports of toxicity with IV paracetamol administered at therapeutic doses in low risk infants were identified in recent published literature or TGA reports since the introduction of IV paracetamol in Australia. The majority of reported toxicity cases in infants <10 kg were due to administration of inadvertent 10-fold overdoses. The strength of IV paracetamol (10 mg/mL), with confusion between mg and mL doses, and lack of adherence to key safe paediatric prescribing recommendations were identified as major contributing factors to paediatric medication errors and associated toxicity. A comprehensive, evidence-based guidance on the appropriate and safe use of IV paracetamol was developed, including a dose recommendation of 15mg/kg/dose in children >3 months.

Discussion: Recent changes to the Australian PI were based on incomplete information. Although NSW TAG's dose recommendations are now off-label, they are justified given the comprehensive evaluation of the best available evidence, input from a multidisciplinary expert clinician panel and development following an agreed systematic evaluation process for off-label uses.

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Does worsening renal function lead to worse long term outcomes in RAAS inhibitor treated patients with left ventricular systolic dysfunction? A meta-analysis of 20,573 patients.

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Introduction. Impaired renal function is associated with worse clinical outcomes in patients with left ventricular systolic dysfunction (LVSD) and heart failure (HF). Renin-Angiotensin-Aldosterone System (RAAS) inhibitors provide clinical benefit in these settings and often worsen renal function.

Aims. To investigate whether worsening renal function (WRF) in patients exposed to RAAS inhibitors predicts a worse prognosis or merely reflects the pharmacological action of the drug on the kidney.

Methods. We performed a meta-analysis of all RAAS inhibitor LVSD trials reporting on outcomes according to WRF (as per individual study definition) in both active intervention and placebo groups looking at the relative risk (RR) using the Mantel-Haenszel fixed-effects model for mortality and major cardiovascular outcomes.

Results. Five major studies (RALES, EPHESUS, SOLVD, SAVE, Val-HeFT) contributed, with 20,573 patients. In placebo treated patients, WRF (n=990) was associated with increased all-cause mortality compared with no WRF (n=9304, RR 1.52, 95% CI 1.37-1.69, p <0.00001). In RAAS inhibitor treated patients, WRF (n=1374) was also associated with increased all-cause mortality compared with no WRF (n=8905, RR 1.22, 95% CI 1.10-1.36, p = 0.0003). Subgroup analysis showed the difference in all-cause mortality between the placebo and RAAS inhibitor groups for WRF was statistically significant (p=0.004). WRF was associated with increased cardiovascular mortality and the combined end-point of cardiovascular mortality/HF hospitalization. HF hospitalization was increased with WRF in placebo only.

Discussion. WRF portends a poor prognosis in LVSD patients, even in those receiving RAAS inhibitors. These findings support preservation of renal function as a key therapeutic goal in the management of patients with LVSD, even in patients treated with RAAS inhibitors.

Oral Abstracts

Dipeptidyl peptidase-4 inhibitors and cardiovascular outcomes: A meta-analysis of randomized clinical trials.

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Introduction: The association between glucose-lowering in subjects with diabetes mellitus and major cardiovascular (CV) outcomes is weak and some hypoglycaemic agents are associated with fluid retention/heart failure. Dipeptidyl peptidase-4 inhibitors (DPP-4i) are a new class of oral hypoglycemic agent.

Aims: We undertook a systematic review to appraise the CV safety and efficacy of these agents.

Methods: A comprehensive search for published and unpublished prospective trials comparing DPP4i with placebo and active comparator was performed. Trials were eligible for inclusion if they reported all-cause mortality rates (at a minimum), recruited minimum 100 patients and minimum follow-up 24 weeks. We performed a meta-analysis of the relative risk (RR) using the Mantel-Haenszel fixed-effects model for mortality and major CV outcomes.

Results: 59 trials met inclusion criteria, enrolling 36,620 patients with mean follow-up of 46.7 weeks. When DPP4i were compared with placebo, there was no difference in all-cause mortality (n=40569, RR 1.04, 0.93-1.16), no difference in CV mortality (n = 39,278, RR 0.97, 0.85-1.11), no difference in MI (n=39,894, RR 0.96, 0.85-1.08), no difference in stroke (n=32,881, RR=0.99, 0.82-1.20) but a statistically significant increase in HF hospitalizations was seen (n=24,111, RR 1.21, 1.03-1.42). Compared with active comparator, there was no difference in all-cause mortality (n=21,134, RR 0.63, 0.38-1.06), no difference in CV mortality (n = 20,672, RR = 0.95, 0.40-2.25), no difference in MI (n = 19,080, RR 0.74, 0.47-1.17), no difference in stroke (n = 12,866, RR 0.57, 0.29-1.11) and no difference in HF hospitalization (n=9,815, RR 0.98, 0.44-2.18).

Discussion: Treatment with DPP-4i compared with placebo shows a nominally statistically significant trend towards increased risk of HF hospitalization. A neutral effect on other outcomes was observed compared to placebo or active comparator. It will be important to see if increased HF hospitalization is observed in other large-scale CV outcome studies with these agents.

Assessment of medication use in Australian prospective longitudinal cohort studies: a missed opportunity

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Introduction. Prospective longitudinal cohort studies of health and aging provide an opportunity for assessing the prevalence and factors associated with medication use in older Australians.

Aims. To identify the medication-related data collected within Australian prospective cohort studies and to examine the potential to apply a range of explicit medication-related assessment tools.

Methods. A review of peer-reviewed literature published from 2000 to 2013 was conducted, using Medline, to identify Australian prospective cohort studies that included medications as a component of data collection. Studies were included if the data collection tools could be obtained from within the public domain. A literature review was conducted to identify explicit medication-related assessment tools; the information required to apply each tool was assessed and compared with the information obtained in each cohort study.

Results. Eight prospective cohort studies were included. Six studies collected a comprehensive list of participant-reported prescribed medication names; two studies collected medication strength; two collected daily dosage; two collected participant reported indication and three collected the duration of treatment. Six studies were identified to have obtained participant consent to supplement data with PBS data. Thirteen medication-related assessment tools were included; relating to comorbidity (n=3), inappropriate prescribing (n=7) and other outcomes (n=3). Seven studies have the potential to enable application of comorbidity assessment tools. Fewer studies had the potential to utilise the range of 'potentially inappropriate medication' screening tools; for example, two studies could fully apply the BEERS criteria. Three studies had the potential to utilise The Medication Regimen Complexity Index (n=5) and three studies the Drug Burden Index.

Discussion. Opportunities exist within Australian cohort studies to improve understanding of medication prescribing, usage and health related outcomes; these opportunities have infrequently been maximised. A 'minimum data-set' of medication-related data should become the standard for incorporation in prospective longitudinal cohort studies of health and aging.

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Optimal Sampling of Antipsychotic Medicines: A Pharmacometric Approach

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Introduction. Antipsychotic medicines display wide variability in response. Identifying the sampling time points that maximize pharmacokinetic (PK) information is important for clinical pharmacology studies and therapeutic drug monitoring (TDM).

Aims. The aim of this study was to determine the optimal sampling time-points and windows to determine clinical PK parameters (AUC, CL) of antipsychotic medicines.

Methods. This study utilised previous population PK models of the antipsychotic medicines aripiprazole, clozapine, olanzapine, quetiapine and risperidone. D-Optimality was utilised to identify time-points which predicted the pharmacokinetic parameters when the drug was at steady-state (SS). Monte Carlo Simulation (MCS) was used to simulate 1000 patients with variability in PK parameters and obtain concentration time-points. Forward stepwise regression analysis was then used to determine the most predictive time-points of the AUC for each drug. Standard two stage population approach (STS) with MAP-Bayesian estimation was utilised to compare AUC_{0-tau} obtained between the optimal sampling and linear regression time-points. Coefficient of variation (CV%) and pearson's correlation coefficient were utilised to compare various sampling strategies for each of the drugs.

Results. Three optimal sampling time points were identified for each antipsychotic medicine. For aripiprazole, clozapine, olanzapine, quetiapine and ziprasidone the CV% of the apparent clearance using the optimal sampling strategies was 19.5, 12.6, 10.3, 17.1 and 10.7, respectively; Using the MCS and linear regression approach to predict AUC_{0-tau}, the recommended sampling strategies included four samples (aripiprazole) (CV%, r^2) (22.9, 0.80), three samples (clozapine) (7.0, 0.97), three samples (olanzapine) (7.7, 0.95), four samples (quetiapine) (6.3, 0.93) and three samples (ziprasidone) (6.6, 0.97). Aside from clozapine, trough concentrations performed poorly when predicting AUC_{0-tau}. The STS approach demonstrated excellent correlations and accuracy in estimation of AUC_{0-tau}.

Discussion. This analysis provides important sampling information for TDM and clinical studies investigating antipsychotic medicines. The translational capacity of pharmacometrics in clinical practice is also highlighted.

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The performance of cystatin C- and creatinine-based eGFR equations for predicting gentamicin clearance

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Introduction. We have previously demonstrated that of the commonly used creatinine-based estimated glomerular filtration rate (eGFR) equations, the CKD-EPI equation, corrected for body surface area (BSA) provided the best estimate of gentamicin clearance [1]. Cystatin C (CysC) is a renal function biomarker with a shorter t½ than creatinine (Cr). CysC-based eGFR equations may therefore provide better estimates of gentamicin clearance.

Aims. To compare the performances of the CKD-EPI Cr- and CysC-based eGFR equations for predicting gentamicin clearance.

Methods. The bias and imprecision of the $CKD-EPI_{Cr}$, $CKD-EPI_{CysC}$, and $CKD-EPI_{Cr+CysC}$ equations [2] for predicting gentamicin clearances, were assessed in 260 patients treated with gentamicin during 2012-2013. The reference gentamicin clearance was calculated using post-dose plasma concentrations in TCIWorks.

Results. The $CKD-EPI_{Cr+CysC}$ equation had the highest percentage of estimates within 30% of the reference gentamicin clearance (70%, $Q(2) = 11.8$, $P = 0.003$) and lowest root mean square error (95% CI) of 29 (26-32) mL/min of the three equations for the entire cohort. There was no significant improvement in the performances of the equations with the exclusion of 41 patients with abnormal thyroid function tests or steroid co-prescription at the time of the index gentamicin dose. Of the remaining 219 patients, removal of BSA normalisation (i.e. converting from units of $mL/min/1.73m^2$ to mL/min) improved the performances of all eGFR equations ($P \leq 0.003$) in the subgroup with body mass indices (BMI) < 18.5 or ≥ 30 kg/m^2, but not those with BMI 18.5 to < 30 kg/m^2.

Discussion. The $CKD-EPI_{Cr+CysC}$ equation provided the best estimate of gentamicin clearance. If used for guiding gentamicin dosing, the CKD-EPI equations should be adjusted for individual BSA at the extremes of body size, to improve estimation of gentamicin clearance.

[1] Chin PKL, Florkowski CM, Begg EJ (2013) Ann Clin Biochem (in press)
[2] Inker L et al (2012) N Engl J Med 367: 20-9.

Oral Abstracts

Patient perspectives' regarding long term warfarin therapy and potential transition to new oral anticoagulant therapy

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Introduction. The potential for new oral anticoagulants (NOACs) to replace warfarin therapy has been recently receiving considerable attention. However, patient perspectives towards these therapeutic advances and the factors that influence these are unknown.

Aims. To explore commonly perceived disadvantages of warfarin therapy and attitudes towards potential transition to NOACs.

Methods. A short quantitative survey was conducted with patients in the community on long-term warfarin therapy. Participants were recruited while attending point of care INR management in the Southern Adelaide metropolitan area. Analyses were conducted using SPSS.

Results. Surveys were completed by 188 participants (93% response rate; 57% male; mean age 76 ± 11.5 years; mean duration of warfarin therapy was 6 ± 5 years). Generally patients were not critical of warfarin therapy; 74% were satisfied or very satisfied with their current therapy. Only 7% disliked any aspect of INR monitoring, while 41% actively appreciated the reassurance it provides. The removal of INR monitoring was only considered an important benefit of transition to NOACs by 21%, while 37% suggested that the lack of regular testing was an important deterrent to transition. Almost half (47%) viewed the lack of an antidote for NOACs as a deterrent. Females were more concerned about transition to NOACs than males (n=180; p=0.014), while the older patients were the less concerned they were about both current and future therapies (n=184; p<0.001).

Discussion. This study suggests that most community-based patients do not perceive warfarin therapy as the significant burden that health professionals suggest, with many actually having a positive attitude towards INR monitoring. Age and sex may be significant factors affecting an individual's desire to transition to NOACs. This information has potential clinical applications for practitioners considering future management of patients currently on long-term warfarin therapy.

Development of indicators for quality use of medicines (QUM) in acute mental health care.

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(introduced by Madlen Gazarian, The University of New South Wales, Sydney, NSW).

Introduction. Improvements in QUM, a core component of Australia's National Medicines Policy, have the potential to reduce morbidity and mortality improving the overall health of Australians. A lack of tools to measure QUM in acute mental health has been recognised.

Aims. To develop indicators to measure QUM processes in acute mental health care.

Methods. A literature search and consultation process was conducted to identify existing Australian and international indicators. A multidisciplinary Expert Advisory Group (EAG), comprising individuals with clinical and therapeutics expertise, reviewed 32 identified indicators, considered their applicability to Australian practice and options for new indicators. Sixteen indicators were then considered from the perspectives of evidence gaps, impact of improvement, ease of measurability and hospital practitioner control with 11 having potential for development. Five indicators were piloted: prescription of 'when required' psychotropics, lithium monitoring, provision of verbal and written information of newly initiated medications, metabolic monitoring of antipsychotics and antipsychotic polypharmacy. Accompanying data collection tools were developed. The indicators were piloted in hospitals of varying demographics across three states. Sites provided collated results and feedback on the measurability, feasibility and relevance of each indicator.

Results. To date, results have been received from 3 of 18 field testing hospitals. Results varied significantly and provide a baseline upon which sites can test the effectiveness of improvement strategies. Feedback reported the indicators were valid, useful, measurable and relevant. Following EAG review of all results, the indicators and data collection tools will be finalised and made freely available by early 2014.

Discussion. QUM processes in acute mental health care are in the developmental phase in many Australian hospitals. These indicators will be aspirational to drive practice change. Accompanying data collection tools assist with identification of systems gaps, enabling focussed practice improvement. Indicator measurement will assist organisations to demonstrate their performance against accreditation standards.

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The cardioprotective effects of stevia and verapamil in hypertensive rats

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Introduction: Hypertension induces significant damage to the heart, blood vessels and kidney and left untreated, promotes the development of cardiac arrhythmias and heart failure. Stevia rebaudiana Bertoni, a naturally sweet herb, has been used for several hundred years as an anti-diabetic and cardiotonic in South America. In limited human and rat trials, stevia reduced hypertension, improved insulin-sensitivity and decreased blood lipids following hypertension and diabetes. Aims: The current study aimed to identify any cardioprotective benefits of stevia in reversing and/or preventing further cardiovascular damage in an animal model of hypertension compared to the calcium channel antagonist verapamil. Methods: We investigated the effects of chronic administration of stevia (200 mg/kg/day; p.o. for 8 weeks) and verapamil (4 mg/kg/day; p.o. for 8 weeks) on 8 week-old WKY (control) and SHR (spontaneously hypertensive rats). Results: Stevia treatment reduced hypertension and prevented maladaptive left ventricular changes and improved systolic and diastolic function in the SHR. This was accompanied by a reduction in the cardiac action potential duration by stevia which reduced the risk of an arrhythmia. Verapamil decreased the hypertension and improved systolic and diastolic function. Positive changes in reactive oxygen species and NO were observed following both treatments. Discussion: In conclusion, this study established that stevia can prevent cardiovascular remodelling following hypertension with putative mechanisms thought to be through multiple pathways such as calcium channel blockade, increased NO synthesis and a reduction in oxidative damage. Verapamil was also shown to be effective in this model acting as an anti-inflammatory, antioxidant and NO promoting treatment in addition to its primary pharmacological role.
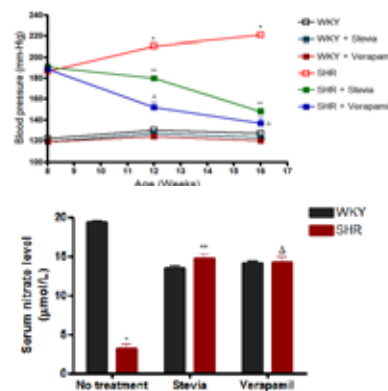
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A CXC-motif receptor 2 (CXCR2) antagonist, SB225002, does not reduce renal fibrosis or systolic blood pressure in deoxycorticosterone-induced hypertension in mice

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Introduction. Leukocyte infiltration into the kidneys during hypertension leads to renal inflammation, fibrosis, and disruption of the pressure-natriuresis relationship. Leukocyte trafficking is regulated by chemokines, which bind to receptors on leukocytes to induce their migration.
Aims. To determine: (1) which chemokines are upregulated in the kidneys in a mouse model of hypertension; and (2) whether pharmacological antagonism of relevant chemokine receptors ameliorates renal fibrosis and blood pressure (BP).
Methods. Hypertension was induced in mice by uninephrectomy, subcutaneous treatment with deoxycorticosterone acetate (DOCA; 2.4 mg/d), and replacing drinking water with 0.9% saline. In some mice, a CXCR2 antagonist, SB225002 (2 mg/kg/day, i.p.) or vehicle (1% DMSO/methylcellulose) was administered for 3 days prior to uninephrectomy and throughout the 21-day treatment period. Systolic BP was monitored by tail cuff for 21 days; mice were killed, and kidneys harvested.
Results. PCR arrays (n=3) and Taqman qPCR (n=8) demonstrated that chemokines CXCL5, CXCL2 and CXCL1 were upregulated in kidneys of DOCA/salt- versus saline-treated mice (P<0.01). The receptor for these chemokines, CXCR2, tended to be upregulated by ~two-fold, although this change did not reach statistical significance (P>0.2). Immunohistochemistry revealed CXCR2-positive cells in the adventitia of renal arteries and interstitial spaces between renal tubules. Treatment with SB225002 failed to prevent DOCA/salt-induced infiltration of CXCR2-bearing leukocytes into the kidney (FACS), increased systolic BP (148±12 vs 148±19 mmHg in vehicle- and SB225002-treated groups, respectively; n≥5; P>0.05) or renal collagen deposition (picrosirius red).
Discussion. These findings imply that CXCR2 may not be a viable target for future therapies to prevent renal inflammation in hypertension.

Oral Abstracts

Anti-inflammatory actions of annexin-A1 peptide Ac[2-26] after myocardial reperfusion injury in mice *in vivo*

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Introduction. Myocardial reperfusion is important to attenuate damage following myocardial infarction (MI). However, this elicits additional ischaemia-reperfusion (I-R) injury, contributing to morbidity and mortality.

Aims. To determine whether annexin-A1 mimetics Ac[2-26] or the small molecule non-peptide Agonist 43 (Ag43) protect against inflammation, myocardial and potential remote lung injury, after 48h myocardial I-R.

Methods. Male C57/bl6 mice (30±0.4g) were subjected to sham (n=6) or 1h coronary artery occlusion (n=8/grp), under ketamine (100mg/kg)-xylazine (20mg/kg) i.p. anaesthesia, with 48h reperfusion. Ac[2-26] (1mg/kg i.v.), Ag43 (50mg/kg i.p.) or vehicle were administered daily on reperfusion. Systemic inflammation was assessed by total and differential white blood cell counts. Myocardial damage was assessed by enumeration of macrophage infiltration (CD68^{+} fluorescence) and qualitative assessment of morphology (H&E score). Lung injury was assessed by tracheal hyperresponsiveness (myograph) and pulmonary oedema (wet:dry lung weight).

Results. Myocardial I-R induced systemic and cardiac inflammation after 48h, but no lung injury was evident. Ac[2-26] and Ag43 both decreased cardiac injury. Ac[2-26] also attenuated the increased serum neutrophils and macrophage infiltration (Table, [*]$P<0.05$ vs sham; [#]$P<0.05$ vs vehicle I-R, unpaired *t*-test; ND, not determined).

RESULTS (48h after I-R *in vivo*)			saline vehicle		PBS/Tween vehicle	
		Sham	I-R	I-R+Ac[2-26]	I-R	I-R+Ag43
Myocardial injury	Morphological injury score (0-5)	1.6±0.2	3.7±0.3[*]	2.9±0.3[*#]	4.0±0.3[*]	2.3±0.4[#]
	Macrophage infiltration (x10^{3}/section)	0.4±0.1	3.8±0.6[*]	2.5±0.3[*#]	4.3±0.6[*]	3.0±0.5[*]
Remote lung injury	Oedema (wet:dry weight)	4.1±0.2	4.1±0.1	4.3±0.1	4.2±0.1	4.4±0.1
	Methacholine pEC[50]	6.4±0.2	6.1±0.1	5.9±0.2	ND	ND
White blood cells (n=4/grp)	Total (x10^{6}/ml)	6.0±0.5	8.5±0.7[*]	7.5±0.4	8.1±1.5	7.9±0.7
	Neutrophils (x10^{6}/ml)	1.6±0.6	2.8±0.1	1.9±0.3[#]	2.7±0.6	2.0±0.3
	Monocytes (x10^{5}/ml)	1.3±0.3	2.6±0.5	2.0±0.5	2.0±0.9	3.8±0.4
	Lymphocytes (x10^{6}/ml)	4.3±0.4	5.5±0.7	5.4±0.3	5.1±0.9	5.6±0.7

Conclusion. These results support further investigation of Ac[2-26] to improve systemic and cardiac outcomes after MI.

Prostacyclin signalling boosts NADPH oxidase 4 in the endothelium promoting cytoprotection and angiogenesis.

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Introduction: Prostacyclin (PGI[2]) released from the vascular endothelium plays an important role in vasodilatation and thromboresistance, and has long been suspected to protect cell survival in the vasculature. How it does so has never been clear. Previously, we and others have shown that NADPH oxidase type 4 (Nox4) improves endothelial functions and promotes angiogenesis *in vitro* and *in vivo*, but it was not known how to boost Nox4 therapeutically to exploit its protective functions in the vasculature.

Aim: To investigate the role of PGI[2]-induced Nox4 in angiogenesis.

Methods: Nox4 gene expression and cell signalling pathways were measured by real time PCR and Western blot analysis in endothelial cells respectively. Hydrogen peroxide (H[2]O[2]) was measured by Amplex red assay. Apoptosis and cell proliferation was measured by caspase3/7 activity and Alamar blue assay respectively. Tubulogenesis was carried out using Matrigel assay. *In vivo* angiogenesis was performed using subcutaneous sponge model.

Results: PGI[2] stable analogue cicaprost increases expression of Nox4 (n=4; p<0.01) in human endothelial cells. Cicaprost enhanced phosphorylation of cAMP response element-binding (CREB) via PKA-dependent pathway and either treatment with PKA inhibitor or over-expression of mutant CREBM1 attenuated cicaprost-induced Nox4 expression suggesting Nox4 up-regulation via cAMP/PKA/CREB pathway. Cicaprost-induced Nox4 and H[2]O[2] production were blocked by Nox4 gene silencing. Importantly, up-regulation of Nox4 by cicaprost protects endothelial cells from apoptosis and enhances their proliferation and angiogenic responses (n= 8; p<0.01), all effects being substantially decreased by Nox4 gene silencing (n= 8; p<0.01). Finally, cicaprost enhanced the blood vessel formation into subcutaneous sponges as indicated by increase in haemoglobin content (n=4; p<0.01) in C57BL/6 mice. This response to cicaprost was substantially reduced (n=4; p<0.01) in Nox4 silencing mice.

Conclusion: This is the first evidence of PGI[2]-mediated up-regulation of Nox4, which opens up a novel strategy to enhance endothelial cell functions in cardiovascular diseases.

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The concomitant coronary vasodilator and positive inotropic actions of Angeli's salt in the intact rat heart are mediated by nitroxyl and soluble guanylyl cyclase-dependent mechanisms.

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Introduction. The NO redox sibling nitroxyl (HNO) elicits soluble guanylyl cyclase (sGC)-dependent vasodilatation. HNO has high reactivity with thiols (unlike NO), which contributes to HNO-enhanced left ventricular (LV) function.

Aims. The present study tested the hypothesis that the concomitant vasodilatation and inotropic actions induced by the HNO donor, Angeli's salt (sodium trioxodinitrate), are sGC-dependent and sGC-independent, respectively.

Methods. Haemodynamic responses to Angeli's salt (10 pmol - 10 μmol), alone and in the presence of scavengers of HNO (L-cysteine, 4 mM) or NO (hydroxocobalamin HXC, 100 μM) or selective inhibitors of sGC (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one ODQ, 10 μM), calcitonin gene-related peptide (CGRP) receptors (CGRP[8-37], 0.1 μM) or voltage-dependent potassium channels (4-aminopyridine 4-AP, 1 mM) were determined in male rat isolated hearts.

Results. Angeli's salt elicited concomitant, potent concentration-dependent increases in coronary flow and LV systolic and diastolic function. Both L-cysteine and ODQ caused a rightward shift in the dose-response curve of each of these effects, implicating HNO and sGC in both the vasodilator and inotropic actions of Angeli's salt. In contrast, neither HXC, CGRP[8-37] nor 4-AP affected Angeli's salt actions.

Discussion. These data suggest that both the vasodilator and the inotropic actions of Angeli's salt are mediated by L-cysteine-sensitive, HNO/sGC-dependent mechanisms, and represent the first evidence that sGC contributes to the inotropic and lusitropic action of HNO in the intact heart. Thus, HNO acutely enhances LV contractile function and LV relaxation, whilst concomitantly unloading the heart, potentially favourable properties for the failing heart.
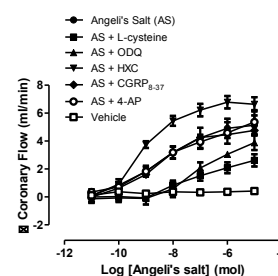
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Inflammatory cell composition in the brain associated with functional outcome following cerebral ischemia

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Inflammatory cells may contribute to secondary brain injury following cerebral ischemia. The C57Bl/6 mouse strain is known to exhibit a Th1-prone, pro-inflammatory type response to injury, whereas the FVB strain is relatively Th2-prone, or anti-inflammatory, in its immune response (Mills et al, 2000). We tested the hypothesis that stroke outcome is more severe in C57Bl/6 than FVB mice. We compared functional outcomes and quantitated immune cell numbers in brains of the two strains of mice following stroke. C57Bl/6 and FVB mice (n=127) were subjected to sham surgery or 1 h occlusion of the right middle cerebral artery followed by 23 h of reperfusion. C57Bl/6 mice displayed greater functional deficits than FVB mice after stroke, as assessed by neurological scoring and hanging grip test (n=28-34, P<0.01). Total numbers of CD45^{+} leukocytes tended to be larger in the brains of C575Bl/6 than FVB mice. Moreover, there were marked differences in the composition of leukocyte types present in the ischemic hemispheres of the two mouse strains after stroke. Compared with sham-operated mice, fold-increases in cell numbers after stroke in C57Bl/6 vs FVB mice (n=5-8) were as follows: total leukocytes, 3.8 vs 3.4; neutrophils, 6.7 vs 96.9; CD3^{+} cells, 3.1 vs 1.6; CD4^{+} cells, 4.7 vs 1.9; CD8^{+} cells, 0.8 vs 2.8; B cells, 2.8 vs 1.6; monocytes 6.0 vs 7.8; macrophages, 2.2 vs 7.9; dendritic cells, 3.5 vs 4.8. The different inflammatory cell environment of the ischemic brain may have contributed to the differential functional outcomes of C57Bl/6 and FVB mice following stroke.

Mills et al (2000) J Immunol 164, 6166-6173.

Oral Abstracts

Signalling profiles and changes in gene expression produced by serelaxin in human vascular cells

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Introduction. The phase III clinical trial, RELAX-AHF, demonstrated that infusion of serelaxin, the recombinant form of the hormone H2 relaxin, over 48 hours improved short- and long-term clinical outcomes in patients with acute heart failure (Teerlink et al. 2013). However, the precise mechanism(s) associated with its cardioprotective actions in humans are poorly understood.

Aims. This study examined the short and long-term effects of serelaxin in cells of the human vasculature endogenously expressing the serelaxin receptor, RXFP1.

Methods. Various cellular signaling assays were utilized to examine the signal transduction mechanisms of serelaxin.

Results. Radioligand binding showed cell surface RXFP1 expression in human umbilical vein endothelial (HUVECs) and smooth muscle cells (HUVSMCs), human umbilical artery smooth muscle cells (HUASMCs) and human cardiac fibroblasts (HCFs), but not in human umbilical artery endothelial cells (HUAECs). In venous cells (HUVECs, HUVSMCs), serelaxin increased cAMP and cGMP accumulation and ERK1/2 phosphorylation (pERK1/2) and the concentration-response curves (CRCs) were bell-shaped. Similar CRCs for cGMP and pERK1/2 were also seen in HCFs, whereas in HUASMCs, serelaxin increased cAMP, cGMP and pERK1/2 with conventional sigmoidal CRCs. Almost all serelaxin responses involved inhibitory G proteins (Gα_i) and PI3 kinase (PI3K). Longer-term serelaxin exposure increased the expression of neuronal nitric oxide synthase (nNOS), vascular endothelial growth factor (VEGF), endothelial receptor type B (ET$_B$) and gelatinase levels, but its effects were more robust in venous cells.

Discussion. Serelaxin signaling was stronger in venous than in arterial cells and that the bell-shaped CRCs that are a hallmark of serelaxin signaling *in vitro*, *in vivo* and clinically, are only observed in venous cells and fibroblasts.

Teerlink, J.R. et al., 2013. Serelaxin, recombinant human relaxin-2, for treatment of acute heart failure (RELAX-AHF): a randomised, placebo-controlled trial. *The Lancet*, 381(9860), pp.29–39.

A novel mechanism of beta2-adrenoceptor-stimulated biogenesis in skeletal muscle

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Introduction. Skeletal muscle plays an integral role in maintaining glucose homeostasis, and skeletal muscle biogenesis is therefore an attractive target for the treatment of metabolic diseases including obesity and diabetes. Exercise promotes noradrenaline release from sympathetic nerve endings located near skeletal myocytes and bind to β_2-adrenoceptors (ARs), where their activation increases glucose uptake (Nevzorova et al., 2006) and mitochondrial biogenesis (Miura et al., 2007) through a cAMP dependent mechanism. While the β_2-AR is the primary AR subtype expressed in skeletal muscle fibres, a traditionally "β_3-specific" ligand, BRL37344, is able to increase glucose uptake via β_2-ARs with negligible effects on cAMP production (Nevzorova et al., 2006), which might provide a novel mechanism to target therapeutically.

Aims. To investigate whether two β-AR ligands, isoprenaline (ISO) that increases cAMP levels, and BRL37344 that has negligible effects on cAMP levels, have differential abilities to promote skeletal muscle biogenesis.

Methods. Using rat L6 skeletal muscle cells, we have measured cAMP levels, glucose uptake, extracellular acidification rate (ECAR) and O$_2$ consumption (mitochondrial respiration) following ISO and BRL37344 treatment.

Results. ISO concentration-dependently stimulated cAMP production (pEC$_{50}$ 8.5±0.2, n=10) and glucose uptake (pEC$_{50}$ 6.8±0.4, n=3), while BRL37344 stimulated glucose uptake (pEC$_{50}$ 6.1±0.3, n=4) with negligible effects on cAMP production (n=10). ISO promoted an increase in ECAR (pEC$_{50}$ 8.4±0.5) and a decrease in O$_2$ consumption (8.0±0.9, n=7), whereas BRL37344 produced negligible effects on ECAR or O$_2$ consumption (n=6).

Discussion. Our results confirm the presence of a novel non-cAMP mediated mechanism of glucose uptake exemplified by BRL37344, however only ISO was able to affect mitochondrial activity, appearing to push L6 skeletal muscle to consuming glucose through glycolysis.

Miura S et al (2007) Endocrinology *148*: 3441–8
Nevzorova J et al (2006) British Journal of Pharmacology *147*: 446–54.

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Calcium regulation of cell functions: From basic principles to therapeutic targets

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Intracellular Ca^{2+} signaling regulates a multitude of cell functions including contraction, secretion, synaptic plasticity, and immune responses. There are many potential molecular targets for drug development in conjunction with the cell functions regulated by Ca^{2+} signals. For example, pharmacological interference with the Ca^{2+} signaling in the heart and blood vessels by calcium antagonists has been utilized for the treatment of hypertension and other cardiovascular diseases. Based on our study on the basic principles of Ca^{2+} signaling, we have been searching for new cell functions that are regulated by Ca^{2+} signals in the brain. We found that nitric oxide (NO) induces release of Ca^{2+} from the intracellular Ca^{2+} store through S-nitrosylation of the ryanodine receptor Ca^{2+} release channel in central neurons. Our results suggest that the NO-induced Ca^{2+} release is one of the causal mechanisms of NO-dependent neuronal cell death, and can be a therapeutic target in certain forms of ischemic brain injury. We also found the involvement of Ca^{2+} signaling in traumatic brain injury. In response to brain injury, astrocytes generate Ca^{2+} signals. Our results indicate that the injury-induced Ca^{2+} signaling causes N-cadherin upregulation in astrocytes around the injury site, and that astrocytic N-cadherin upregulation is required for neuroprotection. These new results highlight the pathophysiological significance of Ca^{2+} signaling in the brain, and the molecules involved the signaling pathways may serve as potential therapeutic targets.

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Do ICs regulate spontaneous activity in the prostate?

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Nerve-mediated contractions of the prostate are important in expelling prostatic fluids into the urethra during the emission phase of ejaculation; however the prostate has also been observed to contract spontaneously. The spontaneous contractions are much smaller in magnitude than the nerve-mediated contractions, and are likely to be involved in the mixing of prostatic secretions as well as contributing to basal tone (Exintaris *et al*, 2002). In the gastrointestinal tract, the spontaneous activity is driven by a specialised group of morphologically-distinct interstitial cells of Cajal (ICCs) which act as pacemakers, initiating, maintaining and co-ordinating gastrointestinal motility. ICCs selectively express the proto-oncogene, c-kit, hence antibodies for c-kit have been used to identify pacemaker ICCs within the gastrointestinal and, more recently, urogenital tracts. The identification of PICs in both guinea-pig and human prostates (Van der Aa *et al*, 2004), coupled with extensive electrophysiological data from our laboratory has led to the suggestion that PICs play a fundamental role in regulating the spontaneous electrical activity and contractility that contribute to the overall prostatic tone (Exintaris *et al* 2002, 2006). Since changes in smooth muscle tone are involved in the aetiology of age-dependent prostate-specific conditions such as BPH, knowledge of the electrical properties of the PICs and their interactions with each other, nerves, and the effects of the hormonal environment, and how these factors change with age is of considerable medical importance.

Exintaris et al, J Urol. 2002; 168(1):315-22.
Exintaris et al, Auton Neurosci. 2006; 126-127:371-9.
Van der Aa, Prostate. 2003; 56(4):250-5.

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Interstitial cells in the gastrointestinal system: multifaceted coordinators of motility
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Interstitial cells of Cajal (ICCs) play an integral role in the coordination of gut motility. Not only do they generate and propagate pacemaker activity such as rhythmic slow waves, but are also believed to serve as mechanosensors and regulators of neurotransmission in the gastrointestinal tract. There is a close apposition between enteric nerves and intramuscular ICs in the gastrointestinal tract, including ultrastructural evidence of synaptic specializations at nerve and ICC junctions. In establishing an important role for ICs in modulating neurotransmission by receiving inputs from motor neurons, ICs have been shown to possess a host of receptors for transmitters and other ligands, including muscarinic and VIP receptors. ICs also respond to neuronal nitrergic signalling, indicating they are receptive 'antennae' for both inhibitory and excitatory neurotransmitters in the intestine. Perturbed function of ICCs has been associated with a wide spectrum of gastrointestinal disorders including achalasia, reflux disease, pyloric stenosis, diabetic gastroparesis, intestinal ileus and pseudo-obstruction. An understanding of the role of altered ICC signalling in these conditions may provide critical insights that extend beyond ICCs as simple relays or intermediaries, but may also realise novel targets for disorders of gastrointestinal motility as further neurotransmitter couplings to ICCs are discovered.

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Interstitial cells in the bladder: role in ageing and pathology
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Interstitial cells (ICs) are found in the urinary bladder in a number of species. However, in spite of more than a decade of research, their functional significance is still not clear. ICs appear to exist as a heterogenous population in the bladder, including fibroblasts, myofibroblasts and interstitial cells, which are located within the lamina propria, suburothelially and at the edge/between detrusor smooth muscle bundles, although these cells still elude a clear morphological classification (Drake et al., 2006; Gevaert et al., 2011). Functionally, ICs have been speculated to be involved in generating or regulating electrical activity and phasic contractile activity of the bladder (Andersson & McCloskey, 2013). As such, changes in the properties of these cells may be important in bladder disorders such as detrusor overactivity, and ultimately may prove novel targets for new therapies for such conditions.
Our research has been focussed on the location and functional role of ICs in the rat and pig bladder and the effect of pathological states and ageing/maturation. This presentation will discuss our findings from functional, molecular and immunohistochemical studies of ICs in bladders from these models, and will highlight the current understanding of the potential role of these cells in bladder function.

Andersson & McCloskey (2013) Neurourol. & Urodyn. DOI 10.1002/nau.22465
Drake et al. (2006). BJU Int 97:29–32.
Gevaert et al. (2011) J. Cell Mol. Med. 15:2586–93

Oral Abstracts

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hERG channel activity controls human uterine contraction in labour and this fails in obesity

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In human uterine smooth muscle, contraction amplitude and duration are controlled by an action potential (AP) that possesses a prominent plateau phase. The ionic conductances responsible for determining the duration of this plateau are unknown. The cardiac AP also has a prominent plateau and the current via hERG1 channels plays an important role. Here we obtained human myometrium following caesarean delivery, following informed written consent. We recorded membrane potential and contraction in strips of myometrium, and ion currents in smooth muscle cells isolated from the same tissue samples. hERG protein levels were determined via Western blotting. hERG blockers dofetilide and E-4031 induced a three-fold prolongation of the plateau phase of the AP and contraction. The hERG activator ICA-195574 reduced contraction duration to 53%. The hERG current in isolated myometrial cells had a maximum amplitude of 3.6±0.4pA/pF and was blocked by dofetilide and E-4031. In tissues obtained from women in established labour, dofetilide also increased AP duration but only by 1.3 fold, and the maximum hERG current was reduced. While levels of the α pore-forming hERG subunit remained unchanged, levels of the β auxiliary subunit, which suppresses the hERG current, were increased 2.4 fold during labour. As BMI increased, the effectiveness of dofetilide in prolonging AP duration was enhanced before (r^2=0.89) and during (r^2=0.68) labour, suggesting enhanced hERG expression/activity. Thus, hERG channels suppressed AP duration and contraction amplitude and duration before labour, facilitating quiescence. Then, changes in hERG channel function, via the β subunit, contribute to AP mechanisms that produce the powerful, sustained, well-spaced contractions typical of labour, and failure of this system likely contributes to the poor labour and increased incidence of caesarean delivery in many obese women. These results demonstrate the dynamic contribution of hERG channels, particularly the β subunit, to native smooth muscle cell function in physiology and pathology.

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Faculty-wide adoption of an active learning approach to replace didactic lectures

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Introduction. It is well established that the traditional lecture format has significant limitations, often resulting in a passive learning experience for students. Paradigms such as problem-based learning have been introduced in biomedical education programs to correct these problems, but have created a new set of difficulties, including poor understanding of key foundation disciplines such as pharmacology. "Active learning", in which didactic teaching is replaced at intervals with specific, goal oriented tasks that all students complete, has been shown in some contexts to allow students to construct and test their conceptual models during class.
Aims. For the above reasons, staff at the Faculty of Pharmacy and Pharmaceutical Sciences have committed to adopting an active learning approach, in which all lectures will be replaced by active learning classes using a staged approach over 4 years.
Methods. A pilot phase was completed and evaluated in 2012, in which active learning principles were developed. In 2013, all first year units underwent transition to the active learning approach.
Results. Evaluation of students across 15 units over 2012 and 2013 revealed that the majority of students viewed active learning as superior to conventional didactic lectures with regard to engagement, clarifying misconceptions and depth of understanding. Staff reported increased enjoyment of classes, and analysis of student performance indicated that students performed as well or better on exam questions that were at higher Bloom's levels than previous exams on the same topics.
Discussion. Overall, the first phase of implementation has been highly successful based on student and staff perception and on maintained or improved performance on exams that were more challenging.

Developing Research Competency

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An understanding of research techniques and methodology is an important part of training for undergraduate biomedical students. The benefits of increasing research competency in undergraduate students include a better understanding of lecture content, a higher level of student engagement and inspiring students to continue with further study or seek out a career in research. In addition, an ability to digest and interpret data from scientific papers is beneficial for students who will go on to study medicine, pharmacy or dentistry or seek work in the Pharmaceutical or Biotechnology industries.

I will discuss some of the ways we have introduced more research content into our third year Neuropharmacology course including technique-focused lectures, journal club format tutorials and laboratory-based projects. In an era of rapidly changing technology and reduced 'wet' laboratory time for undergraduate students, incorporating research techniques and methodology into undergraduate courses is paramount to creating graduates with an understanding if how biomedical research is carried out.

Clinical Pharmacologist Specialty Training

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Introduction. Clinical Pharmacology is the CP in ASCEPT incorporating many disciplines. Clinical Pharmacologists are physicians sub specialists and a registered profession in Australia and New Zealand. After completing a medical degree and basic physician training in diagnostics and therapeutics, trainees may enter the speciality training programme. The training is run by a combined ASCEPT RACP committee.

Recent Challenges. There are too few Clinical Pharmacologists in Australasia to meet the demands in teaching and research for academia and in clinical practice and policy and governance for the health system. The formation of a combined ASCEPT RACP training committee chaired by Evan Begg in 2009 used RACP processes to implement a new curriculum and accredit training sites. The ASCEPT clinical weekend, set up in 2010, has been an anchor point for trainee and supervisor interaction. A highlight for me is working with ASCEPT members from all disciplines. The development of IUPHAR position statement on Clinical Pharmacology in Health Care, Teaching and Research in 2010 and subsequently expanded on in a combined IUPHAR, WHO, CIOMS publication provides a robust international reference point for the discipline. Don Birkett played a major role in this.

This symposium brings together teachers from a range of backgrounds and disciplines. Anecdotes as student, teacher and administrator will be used to seed memes and test paradigms.
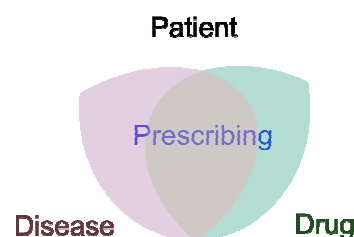
RACP. Advanced Training in Clinical Pharmacology, 2014, available from http://www.racp.edu.au/page/specialty/clinical-pharmacology
IUPHAR, WHO, CIOMS. Clinical Pharmacology in Health Care, Teaching and Research, 2012, available from http://apps.who.int/medicinedocs/documents/s19916en/s19916en.pdf

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Allosteric modulation of muscarinic acetylcholine receptor regulation

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Introduction. Novel selective agonists and allosteric modulators of M_1 and M_4 muscarinic acetylcholine receptors (mAChRs) have been synthesized with significant advantages over conventional orthosteric ligands, including improved subtype specificity and reduced off-target effects. Although these compounds have demonstrated favourable pharmacological characteristics in vitro and in vivo, little evidence is available regarding the effects of such ligands on receptor regulation. The study of receptor regulatory processes triggered upon chronic ligand exposure is particularly important when considering potential therapeutic applications.

Aims. The aim of this study was to characterise the effects of M_1 and M_4 allosteric modulators on mAChR regulation.

Methods. M_1 and M_4 mAChRs were transiently expressed in HEK293 cells, and receptor regulation was measured using ELISA, whole cell radioligand binding and bioluminescence resonance energy transfer.

Results. The orthosteric agonist carbachol induced a strong transient recruitment of β-arrestins to both mAChRs, which was partially dependent on GRK2 kinase activity. The allosteric modulators BQCA and LY2033298 positively modulated β-arrestin recruitment to the M_1 and M_4 mAChRs respectively. Agonist-induced endocytosis of the M_1 mAChR was dependent on β-arrestin recruitment, as mutant M_1 mAChRs with altered β-arrestin recruitment dynamics did not internalise. BQCA and LY2033298 also independently stimulated mAChR internalisation, yet exhibited differential modulation of carbachol-induced mAChR internalisation and subcellular trafficking.

Discussion. Our results show that in heterologous systems, positive allosteric modulators of M_1 and M_4 mAChRs also behave as such with regards to receptor regulation. Such behaviour has important implications when considering the in vivo consequences of prolonged exposure to these two different molecules. Thus, BQCA and LY2033298 could potentially induce receptor internalisation in the absence of ACh and, in the presence of ACh, their effects on receptor activation could be limited by the simultaneous loss of receptors from the plasma membrane.

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Development of an irreversible allosteric ligand for the M_1 muscarinic acetylcholine receptor

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MIPS1262 - irreversible allosteric ligand

Introduction. Potentially all G protein-coupled receptors (GPCRs) contain allosteric binding sites that can be targeted by novel, highly-selective therapeutic agents (May *et al*. 2007). One such GPCR is the M_1 muscarinic acetylcholine receptor (M_1 mAChR); a target of therapeutic interest for the treatment of cognitive deficits (Langmead *et al*. 2008).

Aims. To elucidate the binding site of a M_1 mAChR-selective, orally bioavailable allosteric ligand, BQCA (Ma *et al*. 2009) using irreversible analogues of this molecule. Ultimately, this will more efficiently guide rational drug design of putative clinical candidates.

Methods. Synthetic medicinal chemistry, saturation and competition radioligand binding, ERK1/2 phosphorylation functional assays

Results. The allosteric binding and functional properties of BQCA were preserved to varying extents in all four putative irreversible analogues, and data for one analogue, MIPS1262, suggest that an irreversible interaction with the receptor has been successfully formed.

Discussion. MIPS1262 will serve as a useful structural probe to identify the BQCA binding site, either by analyzing the ligand-receptor complex to determine the amino acid residue involved in the irreversible interaction or, ideally, by co-crystallisation with the M_1 mAChR.
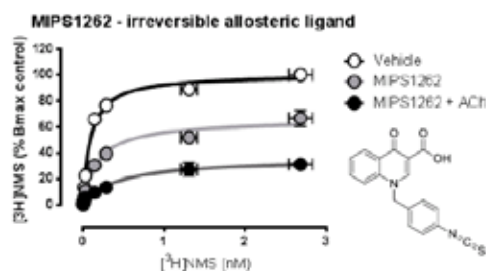
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May, L.T. *et al*. (2007) Ann Rev Pharmacol Toxicol 47:1-51.

Langmead, C.J. *et al*. (2008) Pharmacol Ther 117:232-243

Oral Abstracts

A tyrosine kinase inhibitor blocks PAR₂-mediated pain

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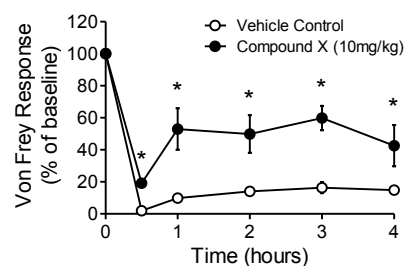
Aims. Protease activated receptor 2 (PAR₂) receptors are expressed on pain-sensing nerves, and can sensitise transient receptor potential (TRP) ion channels to amplify pain. The mechanisms by which this occurs are unknown. We have previously shown that PAR₂ activation in a cell line causes TRPV4 to open ("coupling"). Here we aimed to determine the intracellular signalling mechanisms leading to PAR₂-TRPV4 coupling and investigate the importance of this phenomenon in PAR₂-mediated pain.

Methods. Intracellular calcium levels were measured after PAR₂ activation (with SLIGRL or trypsin) in HEK293 cells either lacking (non-transfected, NT) or stably expressing human TRPV4 (hTRPV4) ion channels. These cells endogenously express PAR₂ receptors. Selective activators or inhibitors were used to pharmacologically investigate the signalling mechanisms leading to PAR₂-TRPV4 coupling. Murine mechanical pain responses were assessed using the von Frey hair technique for applying punctate pressure to the paw after subcutaneous intraplantar injection of the PAR₂ agonist SLIGRL.

Results. In NT HEK293 cells, PAR₂ agonists stimulated a transient increase in intracellular calcium. Expression of hTRPV4 lead to a sustained intracellular calcium increase (coupling), which was inhibited by a selective TRPV4 antagonist (HC067047) and a tyrosine kinase inhibitor (Compound X); but not an alternative tyrosine kinase inhibitor (Dasatinib). Compound X significantly inhibited pain responses following PAR₂ injection ($p<0.05$), which have previously been shown to be mediated by TRPV4.

Discussion. This study demonstrates a role for tyrosine kinases in PAR₂-TRPV4 coupling. Compound X is currently in clinical trials for cancer indications. The ability of Compound X to diminish PAR₂-induced pain also identifies this drug as a potential novel therapy for pain hypersensitivity.

A Structure-Activity Analysis of Biased Agonism at the Dopamine D2 Receptor

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Introduction. Biased agonism offers an opportunity for the medicinal chemist to discover pathway-selective ligands for GPCRs. A number of studies have suggested that biased agonism at the dopamine D2 receptor (D2R) may be advantageous for the treatment of neuropsychiatric disorders, including schizophrenia. As such, it is of great importance to gain insight into the structure activity relationship of biased agonism at this receptor.

Aims. We have demonstrated that both the clinically used antipsychotic aripiprazole and cariprazine, a drug awaiting FDA approval for the treatment of schizophrenia, display a similar bias towards inhibition of cAMP as compared to phoshorylation of pERK1/2 at the D2R. We undertook a structure–activity study to identify the structural determinants that underlie such bias.

Methods. We identified a novel D2R partial agonist *tert*-butyl (trans-4-(2-(3,4-dihydroisoquinolin-2(1H)-yl)ethyl)cyclohexyl)carbamate (MIPS1026). This ligand shares structural similarity to cariprazine yet displays a distinct bias profile. We synthesized a number of derivatives of MIPS1026 with subtle structural modifications, including incorporation of cariprazine fragments. We combined pharmacological profiling using assays to measure pERK1/2 phosyphorylation and intracellular cAMP production with novel analytical methodology to identify and quantify bias.

Results. We have demonstrated that efficacy and biased agonism can be finely tuned by minor structural modifications to the head group containing the tertiary amine, a tail group that extends away from this moiety and the orientation and length of a spacer region between these two moieties. For example, cariprazine displayed a 200-fold bias towards the cAMP pathway. Replacement of the dimethyl urea tail group of cariprazine with a tert-butyl carbamate moiety resulted in a 42-fold decrease in bias towards this pathway.

Discussion. In conclusion, this approach has provided an unprecedented insight into the molecular determinants and SAR of biased agonism at the D2R.

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Brite adipocytes derived from subcutaneous white adipose tissue display enhanced β-adrenoceptor function

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Introduction. The high prevalence of obesity has provoked substantial interest in adipocyte thermogenesis. Whereas classical white adipose tissue (WAT) stores chemical energy, brown adipose tissue (BAT) releases energy as heat, thereby counteracting weight gain. Imaging studies have revealed functional BAT in adult humans, and several papers have demonstrated inducible "brite" (brown in white) adipocytes in animal models and human samples. Brite adipocytes are characterised by expression of the uncoupling protein UCP1, and although they are derived from a white adipocyte lineage, express the brown adipocyte transcriptional co-regulator Prdm16 (Seale et al, 2011). Brite differentiation is induced by multiple stimuli including the PPARγ activator rosiglitazone (Petrovic et al, 2010).
Aims. We determined the effect of rosiglitazone (1 μmol/L) on gene expression profiles and β-AR function in primary mouse adipocytes derived from the stromal vascular fraction of interscapular BAT, subcutaneous inguinal WAT (iWAT) and epididymal WAT (eWAT).
Methods. Gene expression was measured by qPCR, and β-AR function was assessed by determining noradrenaline concentration-response curves for cAMP (αScreen, Perkin Elmer) and oxygen consumption (OCR, Seahorse XF96).
Results. cAMP and OCR responses were absent from control iWAT and eWAT cultures but were markedly induced in rosiglitazone-treated cells, in parallel with increased expression of the β_3-AR. Despite similarities in β-AR function, rosiglitazone-treated iWAT cultures had 17-fold higher expression of UCP1 than the corresponding eWAT cultures. This was not related to the degree of adipocyte differentiation, however iWAT cultures had substantially higher expression of markers of brite thermogenesis, notably Prdm16, Ppargc1a, and Cpt1b.
Discussion. Our data indicate that cells from subcutaneous iWAT undergo rosiglitazone-induced brite differentiation in concert with β_3-AR expression, and thus have the capacity for increased thermogenesis via UCP1 activation.

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Orexin 2 receptor antagonism induces sleep: a novel series of Orexin receptor antagonists with distinct effects on sleep architecture.

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Introduction: Orexin peptides are produced in very discrete populations of hypothalamic neurons and activate two G protein-coupled receptors, OX_1R and OX_2R. The orexin system plays a role in the sleep-wake cycle, feeding and reward seeking. The validity of targeting the orexin system for treatment of sleep disorders has been established clinically, with the dual orexin receptor antagonists (DORAs), Almorexant, Suvorexant, SB-649868 and Filorexant, in primary insomnia. However, the relative contributions of OX_1R and OX_2R to sleep architecture are still debated.
Aim: We initiated a drug discovery program to create both OX_2R selective antagonists and DORAs.
Methods: Following treatment with orexin receptor antagonists, polysomnography was used to evaluate sleep in wild-type, OX_1R, OX_2R and OX_1R/OX_2R knockout (KO) C57BL/6J mice. Locomotor activity following orexin-A administration was used to assess the contribution of OXR to arousal.
Results: OX_2R selective antagonists induced sleep primarily by increasing non-REM (NREM) sleep, whereas suvorexant increased rapid eye movement (REM) sleep. Almorexant dose-dependently increased REM and NREM sleep in C57BL/6J mice. Both, almorexant and orexin A were ineffective in double OX_1R / OX_2R KO mice, thus their actions are mediated by OX_1R / OX_2R only. Orexin A-induced locomotion and sleep induction by almorexant were absent in OX_2R KO mice, but present in OX_1R KO.
Discussion: OX_2R antagonism is sufficient to promote sleep in mice. OX_2R selective antagonists may be beneficial for treating insomnia.

Understanding the spatio-temporal profile of compartmentalised G protein coupled receptor signalling.

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Introduction. Cells endogenously express a variety of different receptors that can activate the same second messenger but with remarkably diverse physiological outcomes. This implies a high degree of organisational constraint in signal transduction. Spatial and temporal compartmentalisation of signalling provides a mechanism for GPCRs to affect numerous aspects of cell control in a specific and orchestrated manner. However, population-based signalling assays lack the resolution to delineate the role of compartmentalised signalling in GPCR function.


Aim. To develop a 'toolbox' of sub-cellular targeted FRET biosensors, with the resolution to measure compartmentalised receptor signalling in single live cells.

Methods. Existing FRET biosensors for different signalling effectors (including ERK1/2[1] and cAMP[2]) were targeted to sub-cellular regions important in GPCR trafficking and regulation, including the cytosol, plasma membrane, endosomal pathways and nucleus. The spatio-temporal dynamics of GPCR signalling were measured using the targeted FRET biosensors for different signalling effectors.

Results. Compartmentalisation of GPCR signalling can be regulated at multiple levels, including by localisation within specific membrane microdomains and by ligand-dependent receptor trafficking following GPCR activation. Disruption of these layers of regulation cause significant changes in the spatio-temporal profiles of signalling, which may translate to dysregulation of physiological outcomes.

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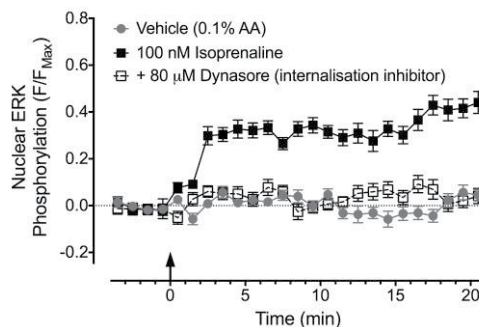
Anti-Proteus activity of some South African medicinal plants: their potential for the prevention of rheumatoid arthritis

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Introduction. Rheumatoid Arthritis (RA) is a chronic inflammatory disorder of the joints which afflicts 0.5 - 1 % of the world's population, with approximately three times as many women affected as men. The causes of RA are poorly understood although it is generally accepted that it is an autoimmune disorder triggered by microbial infection (particularly by Proteus spp.). Whilst there is currently no known cure for RA, a wide variety of herbal remedies are used in traditional African medicine to treat (RA) and inflammation.

Aims. South African plants with a history of ethnobotanical usage were tested for the ability to block the bacterial trigger of rheumatoid arthritis.

Methods. Thirty four extracts from 13 South African plant species with a history of ethnobotanical usage in the treatment of inflammation were investigated for their ability to control two microbial triggers for RA (*P. mirabilis* and *P. vulgaris*). The Artemia nauplii bioassay was used to screen the extracts for toxicity.

Results. Twenty nine of the extracts (85.3 %) inhibited the growth of *P. mirabilis* and 23 of them tested (67.7 %) inhibited the growth of *P. vulgaris*. Methanol and water extracts of *Carpobrotus edulis*, *Lippia javanica*, *Pelargonium viridflorum*, *Ptaeroxylon obliquum*, *Syzygium cordatum* leaf and bark, *Terminalia pruinoides*, *Terminalia sericea*, *Warburgia salutaris* bark and an aqueous extract of *W. salutaris* leaf were effective Proteus inhibitors, with MIC values < 2000 μg/ml. The most potent extracts were examined by RP-HPLC and UV-Vis spectroscopy for the presence of resveratrol. Only extracts from *T. pruinoides* and *T. sericea* contained resveratrol, indicating it was not responsible for the anti-Proteus properties reported here. All extracts with Proteus inhibitory activity were also either non-toxic, or of low toxicity in the Artemia nauplii bioassay.

Discussion. The low toxicity of these extracts and their inhibitory bioactivity against Proteus spp. indicate their potential for blocking the onset of rheumatoid arthritis.