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ORAL ABSTRACTS

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Neuroprotection: Challenges For The 21st Century

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Tomorrow's world will be characterized by an ever-increasing aging population. The central nervous system can be significantly and irreversibly affected by acute injury or by slowly-developing neurodegeneration. The concept of neuroprotection can be explored in this dual context. Traumatic brain injury and spinal cord injury are examples of complex pathologies which have been the target of repeated attempts to protect the nervous system post- injury, but so far, the results in the clinic have been less than satisfactory. It is appropriate to review the pathophysiology concepts and the proposed sequence of events which leads to tissue demise following neurotrauma, and consider the principles governing the present clinical translation. Recent efforts in neurotrauma with compounds such as minocycline, glibenclamide, omega-3 fatty acids (1) or progesterone can be used as an example in order to better understand areas which may still be driven by rather empirical decisions in translation. Neurotrauma outcome is also influenced by a changing demographics; the increased proportion of older patients affected by acute injury increases the challenge of successful protection of a nervous system which is affected by both injury and insidious age-linked degeneration. Concerning neurodegeneration, one of the most important trends in the neurotherapeutics of the future is the temporal shift in the critical period of intervention aimed at aborting neurodegenerative processes. Neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease, which have been historically considered distinct entities, although heterogeneous, are now viewed from a novel angle, influenced by network modelling (2) and with a new understanding of common features such as the non-random propagation of abnormal protein aggregates. Interestingly, such pathological aggregation can be initiated by neurotrauma – thus, the challenge of neuroprotection bridges the acute and the chronic and requires entirely new approaches.

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Orexin receptor antagonism in sleep disorders: preclinical and clinical aspects.

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Orexins A&B (also known as Hypocretins 1&2) are produced in the lateral hypothalamus (LH) and activate two G protein-coupled receptors, OX_1R and OX_2R. The orexin system modulates sleep-wake balance, feeding and reward seeking. Human narcolepsy with cataplexy is due to the absence of LH orexin producing cells. The validity of targeting OX_1R and OX_2R in sleep disorders is established clinically, with dual orexin receptor antagonists (DORAs), Almorexant, Suvorexant, SB-649868 and Filorexant (1). Orexin receptor antagonists specifically block arousal, in contrast to benzodiazepine and Z-drugs, which inhibit neuronal activity. The FDA recently approved Suvorexant for the treatment of insomnia (1,2). However, the relative contributions of OX_1R and OX_2R to sleep architecture are debated. Selective OX_2R antagonists have different effects on sleep architecture from DORAs, whereas OX_1R antagonists do not affect sleep. DORAs decrease wakefulness and increased sleep in WT and OX_1R knockout (KO) mice, but did not affect sleep in OX_2R and double OX_1R/OX_2R KO mice (1,3). OX_2R antagonists increased NREM / REM proportionally, whereas DORAs primarily increase REM. When combined, OX_2R and OX_1R antagonists disrupt balanced sleep by increasing REM at the expense of NREM. Finally, OX_2R antagonists do not perturb normal sleep in mice during the inactive phase, whilst DORAs significantly shifts the balance towards REM (1,3). The altered REM/NREM balance may contribute to adverse effects of DORAs e.g. abnormal sleep behaviours and potential for muscle weakness (1). Although OX_2R antagonism alone appears sufficient to induce and maintain balanced sleep in rodents, clinical evidence is still lacking and awaits proof of concept in patients.

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Allosteric Targeting Of Muscarinic Acetylcholine Receptors

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The muscarinic acetylcholine receptors (mAChRs) are prototypical members of the G protein-coupled receptor (GPCR) superfamily that regulate numerous fundamental processes in both the central and peripheral nervous system (1). In particular, the M_1 and M_4 mAChRs have emerged as exciting drug targets for the treatment of cognitive deficits associated with Alzheimer's disease and schizophrenia, but remain suboptimally targeted due to the very high degree of conservation of the orthosteric, acetylcholine-binding, site across all 5 receptor subtypes. However, we have shown that these (and other) GPCRs possess spatially distinct allosteric sites that provide greater selectivity in modulating receptor function (2). We have now solved high-resolution crystal structures of the M_1 and M_4 mAChRs in complex with the clinically used antagonist, tiotropium, and are using these to understand the structural basis of orthosteric and allosteric ligand interaction with this important class of GPCR. In addition, we have developed chemical biology approaches to enriching allosteric ligand structure-activity studies (3-5) and thus generating new tools that allow us to probe the in vivo consequences of allosteric targeting of the mAChRs. In particular, we have provided proof of concept that combination of an M_1 mAChR positive allosteric modulator with existing antipsychotic medications can yield synergistic efficacy in a number of animal models commonly used to understand different domains of the schizophrenic syndrome. These recent findings can facilitate the development of new mAChR subtype-selective ligands that could prove useful for the treatment of numerous pathophysiological conditions

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Peptides and Reward-Seeking Behaviour

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Introduction. Relapse represents the most difficult clinical problems in treating patients with alcohol and substance use disorders. Increasing our understanding of the brain circuits and chemicals that drug intake and relapse offers the potential for more targeted therapeutic approaches to assist in relapse prevention.
Aims. We sought to unravel brain nuclei where orexin, corticotropin releasing factor (CRF) and relaxin-3 mediate relapse.
Methods. We used abstinence and extinction-reinstatement paradigms to examine the role of specific peptide systems in relapse to reward-seeking.
Results. Orexin, CRF and relaxin-3 all act, and appear to also interact, within circuits mediating cue and/or stress-induced relapse-like behaviour. For example, orexin1 receptors in the ventral tegmental area and prelimbic cortex regulate cue-induced reinstatement of alcohol-seeking in rats; orexin2 receptors within the nucleus incertus mediate stress-induced alcohol-seeking while CRF1 receptors in the ventral tegmental area mediate stress-induced cocaine-seeking. Relaxin-3 acts upon RXFP3 receptors in the bed nucleus of the stria terminalis to regulate stress-induced reinstatement of alcohol-seeking in rats.
Discussion. Multiple neuropeptides act independently, and at a network level, to mediate relapse-like behaviour.

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Using Biased Opioid Agonists To Understand Receptor Signaling In Vivo

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Introduction. Genetically modified mice that lack ßarrestin2 display a number of phenotypes in response to morphine that suggest that it would be beneficial to activate morphine's target, the mu opioid receptor, in a manner that avoids ßarrestin2 interactions. Such behaviours include enhanced antinociception with little tolerance and protection from constipation and respiratory suppression.

Aims. We have developed MOR agonists that activate MOR yet have very little propensity for recruiting ßarrestin2 to the receptor; these are used to test the hypothesis that biasing MOR signalling toward G proteins and away from ßarrestin2 interactions may produce antinociception with less side effects Methods. Compounds have been pharmacologically characterized in vitro and in vivo and compared to morphine.

Results. We find that agonists that bias MOR signalling through G proteins induce robust antinociception that is equipotent or more potent than morphine with no tolerance. Decreases in respiratory measures are modest compared to morphine and only occur at doses exceeding doses required for antinociception. Constipation varies with the compounds, with some showing no constipation and other producing constipation with initial dosing that wanes with chronic administration. Pharmacokinetic and pharmacological selectivity data will also be presented.

Discussion. By developing a relatively large cohort of biased MOR agonists, ranging in potencies and efficacies across signalling platforms, we have the opportunity to evaluate what type of biased agonist may be most useful for discerning how to refine desired physiologies such as antinociception away from adverse effects, such as respiratory suppression. Ultimately, such knowledge may not only be useful in directing our approach to developing opioid analgesics with reduced side effects, but may also point towards experimental strategies for GPCR therapeutics development.

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Has Genotyping Fulfilled Its Hopes to Individualise Drug Therapy?

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Variability in drug response can be due to clinical, environmental or genetic factors. For example, the presence of renal impairment can increase drug exposure increasing the risk of an adverse drug reaction. In such a case, the drug label will usually provide some instructions to reduce the dose so as to normalize drug exposure. Genetic factors can affect both the pharmacokinetics and pharmacodynamics of a drug, which can both reduce efficacy or promote toxicity. Since the birth of the research area of pharmacogenetics in 1957, and more latterly, since the completion of the human genome project, a lot of information on genetic factors affecting drug response has been published. This has led to an increased number of drug labels where pharmacogenetic information is mentioned – for example, of the 517 centrally approved products in the EU, 15% of labels contain pharmacogenetic information. However, this is mostly for information. With the exception of targeted therapies in oncology, there are very few non-neoplastic drugs (abacavir, ivacaftor, eliglustat) where genetic tests are mandated. There are many reasons for this including the difficulties in replicating phenotype-genotype associations, the lack of availability of genetic tests and the perceived costs of testing. Furthermore, while drug exposure which changes as a result of renal or hepatic disease often leads to prescribing information based on PK modelling approaches, a genetic factor which leads to the same change in drug exposure is not treated in the same way. Given the waste that is seen in drug therapy, the fact that drug usage will increase with an increasingly elderly population, and there is a large burden caused by iatrogenic disease, it is encumbent upon all of us to study all factors that lead to variation in drug response, and develop implementation methods that are acceptable to clinicians, the payers and regulators.

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Can PBPK Help To Individualise Drug Dosage?

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The application of physiologically-based pharmacokinetic (PBPK) modelling has come of age in drug development and regulation, reflecting significant advances over the past 15 years in the predictability of key pharmacokinetic parameters from human *in vitro* data and in the availability of dedicated software platforms and associated data bases. With respect to understanding co-variates and variability, focus in applying PBPK has been on anticipating the quantitative impact of drug-drug interactions, age, genetics, racial differences, disease, food effects and pharmaceutical formulation. These extensions of PBPK modelling, along with the incorporation of the PK of biologicals and moves towards linking PBPK to pharmacodynamic (PD) outcome, are clearly of benefit in understanding extremes of risk in different patient populations as part of the process of drug development. Indeed, mechanistic PBPK modelling is the only efficient methodology that can anticipate the combined effects of many patient variables acting simultaneously. The next challenge for PBPK-PD is its direct application in health care, concentrating on the individual rather than the population, as an educational tool and for the provision of computerised, 'point of care' advice on personalised drug dosage. The safe and effective management of multi-drug treatment of the complex patient with multiple diseases and multiple prescribers requires an integrated view of pharmacology and therapeutics. In this context, linking the real patient to his/her 'virtual twin' and a PBPK-PD model in the cloud through a tablet is technically feasible and promises to predict appropriate, rapid individualized/stratified drug dosage, and to avoid undesired complex multiple drug-drug interactions. Practical issues in making this proposition a reality will be discussed including the availability of sufficient patient input data (electronic medical records, demographics, genotypes, co-medication, biomarkers), the availability of a sufficient range of unit dose preparations, physician and Pharma resistance, the relationship of dose prediction (PBPK) to dose adjustment (TDM/adaptive feedback), evidence of cost-benefit for payers and regulatory approval.

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Use Of Microdose Phenotyping To Individualise Dosing Of Patients

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Microdosing of probe drugs for drug metabolising and drug transporters might be a tempting approach to individually assess a patient's actual enzyme and transporter activity. However, linearity of microdose pharmacokinetics must be rigorously analysed because absorption, distribution, metabolism, and excretion of a drug can all be nonlinear and cause differences between the pharmacokinetics of microdoses and regular doses. The CYP3A probe drug midazolam has been proven to exhibit linear pharmacokinetics for dose ranges down to 100 ng orally (1). In this study in healthy volunteers the extent of potent CYP3A inhibition using ketoconazole was nicely predicted with the midazolam microdose, suggesting that this unobtrusive procedure might be suitable for use in patients who are seriously ill and heavily treated. Indeed, when oral midazolam microdoses (3 µg) were administered to hematological patients and CYP3A activity was assessed, patients receiving posaconazole were clearly inhibited shown by an 8.5-fold higher midazolam AUC than matches control patients (2). Kidney insufficiency (GFR<60 ml/min) did not influence midazolam microdose clearance (n=8). Even in patients the use of 4 plasma samples drawn between 2 and 4 h after dosing can be used to predict the midazolam partial metabolic clearance as a measure of CYP3A activity (Siller et al, unpublished). In healthy volunteers it has been shown that CYP3A activity-based dose adaptation can be used to reduce interindividual variability in simvastatin exposure (3). Hence, the basis for the dose individualisation has been set by means of CYP3A activity assessment, which can be extended to a cocktail approach using microdoses. This has the advantage that no drug-drug interactions will occur between the microdosed probe drugs.

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Differential Approach For Dose Individualisation Using Pheno- And/Or Geno-Typing

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Traditionally, therapeutic drug monitoring (TDM) using concentrations as an index of exposure has been used to adjust dosage for drugs with a narrow therapeutic index, mainly to avoid toxicity and/or optimize dosing. Drugs for epilepsy, immunosuppressants and mania are prime examples. However, although the rationale has conceptual merit, there have been very few if any randomized trials to validate their usefulness and cost-effectiveness. Nevertheless, this approach remains the domain of many pathology and clinical pharmacology laboratories. In some cases, a biomarker of response is better, such as the INR for some anticoagulants as it is closer to the therapeutic endpoint. More recently, genotyping has been used, often as a substitute for TDM based on enzyme (for example CYP2D6, TPMT, CYP2C9) polymorphisms, with *TPMT* genotyping the most widely used when azathioprine or 6-MP are prescribed. In some instances, a case can be made for both. For example for warfarin, pre-emptive testing using *CYP2C9*/*VKORC1* genotyping can be used to optimize the initial dose followed by INR monitoring for fine tuning. Similarly, *TPMT* genotyping to avoid bone marrow toxicity through initial dose selection is used extensively but for therapeutic effect, 6-thioguanine nucleotide (the active metabolites) concentrations are sometimes used, especially for Crohn's disease treatment. Finally for tacrolimus, it is essential that concentrations in the blood (not site of action) be optimized and this can be done by *CYP3A5*3* genotyping followed by traditional TDM. It is likely that in the future, a combination of geno- followed by pheno-typing may become a common strategy. Genotyping for initial dose selection and phenotyping for biomarkers of response or toxicity, for optimization. Nevertheless, adoption of such an approach will be highly dependent on clinical champions in the various specialities.

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Pharmacology Education Online - Exploring The Virtual Pharmacology Space

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Introduction. Pharmacology education traditionally was a mix of lectures, tutorials, and laboratory classes, whereas now it is much more of a blend of face-to-face teaching and student-centred study with the latter being supported increasingly by online resources.
Aims. This presentation will explore what online resources are available both to faculty to support their teaching and to students to enable them to learn independent of tutor input.
Results. Various online resources will be described and evaluations of educational effectiveness, where available, will be reported. One significant change to pharmacology education over the last 20 years has been a gradual reduction in the number of laboratory sessions particularly those which investigate the actions of drugs on both *in vivo* and *in vitro* animal preparations. The reduction has been driven by cost, reduced space within the curriculum, ethical objections from students and the availability of computer-based simulations some of which have been shown to be educationally effective (1). Multimedia tutorials, covering numerous pharmacological topics, online formularies, e-books, structured and unstructured repositories of learning objects, MOOCs and even complete online degree courses are now available and accessible via the Internet and having an impact on the way in which pharmacology is taught.
Discussion. Pharmacology education has undergone significant transformation with increasing use of technology and there ate examples of this delivering positive impact on student learning. Student ownership of devices giving them access to online resources has increased dramatically, they are more IT-literate, learn differently, and have different expectations.

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Pharmacology Online For Nurse Practitioners In Urban, Rural, And Remote Areas Of Australia - What Works And What Doesn't

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The first Nurse Practitioner (NP) was appointed in NSW in 2001 and as of March 2015 there were 1,165 NPs authorised in Australia. The challenges facing Australian Higher Education Providers involved in the education of the NPs include not only the wide geographic location of NP candidates but also meeting the needs of a diverse group with varied nursing and clinical backgrounds, differing levels of prior knowledge and widely varying computer literacy.

Delivery solely on-line of a pharmacology course to students in urban, rural and remote areas of Australia has presented the Institution, the e-moderators and the e-learners with multiple challenges that are complex and invariably inter-related. This presentation will explore the many issues that have been faced over a ten year period by each member of the learning triad, what has been 'fixed' and what is still 'broken'. The issues faced have included (1) problems associated with the technology; (2) educational factors such as the continual development/revision/contextualising of on-line teaching and learning materials that promote e-learner self-regulation, the pitfalls and promises of multimedia elements and the challenges of assessment and higher order learning; (3) the skills, knowledge and attitudes of both the e-moderators and the e-learners and (4) the real/perceived 'isolation' within the learning triad in particular geographic isolation of the e-learners from the e-moderators. Higher Education Providers continue to embrace efficiencies in teaching. At the same time there is an exponential growth in the provision of highly flexible learning environments, which enable students to meet their own learning goals at times that are often best suited to work and family commitments. There are still many challenges but perhaps one we have yet to face is on-line *éducation sans frontières*.

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Prescribing Safety Assessment: Assessing The Basic Prescribing Skills Of UK Medical Graduates

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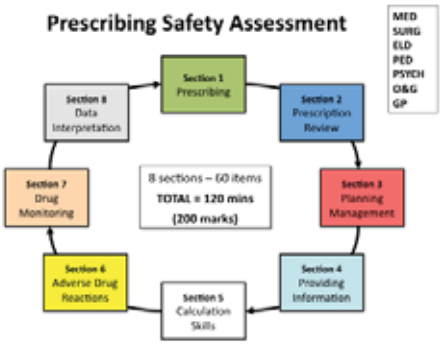
Prescribing is a challenging activity that involves a complex mix of knowledge, judgement and skills. New doctors often express a lack of confidence in writing prescriptions and are often involved in prescribing incidents in hospitals (often because most systems expect them to undertake the majority of the prescribing). A valid and reliable assessment of prescribing competence, separate from an overall assessment of medical knowledge and skill, would have many benefits for clinical governance and patient safety, and would provide a measure of the success of training programmes in therapeutics. Developing and delivering such an assessment presents many challenges. This presentation describes the establishment of the *Prescribing Safety Assessment* in the UK including (i) the building of a novel online authoring, editing, assessment creation and delivery engine, (ii) construction of the assessment, (iii) item development and quality assurance, and (iv) the various regulatory and political hurdles that have to be overcome. It will also present data that has accumulated on performance following piloting and implementation in 2014.



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Blended Learning - Working Without A Safety Net

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Introduction. Blended learning is not really a new teaching and learning approach. It is being used at all educational levels (K-12 to University) as well as in vocational training. Blended learning is usually regarded as a mix of face-to-face classes, on-line work, individual and collaborative learning activities that create a flexible, more active learning experience for students. There have been a number of recent reports in the literature of blended learning being used in the delivery of pharmacy and pharmacology courses.
Aim. Provide an account of the experience of implementing a blended learning approach to delivering pharmacology, parallel to other biomedical sciences, into the pre-clinical year of a graduate entry medical course and highlight the lessons we have learned so far.
Methods. In an 'all-or-nothing' way, the pharmacology content of the preclinical year is being delivered using a flipped classroom model. This entails the material being divided into content delivered prior to class (through the Moodle learning environment, usually in the form of on-line lectures), and that which is addressed face-to-face in class. The pre-class activities are directed towards developing basic knowledge acquisition and comprehension, while face-to-face activities involve short quizzes, and content application and analysis in collaborative student group activities.
Discussion. As others before us have reported, students were initially unsettled in, and resistant to, the flipped classroom despite orientation to this delivery mode. They reported a strong feeling of being lost, not being able to clearly identify learning objectives and having to self-teach content. For staff, the preparation for this delivery mode is very intense. We decided our implementation would be relatively low tech, opting for simple on-line lecture recordings rather than using more sophisticated software. Interestingly, we tend to spend much more time preparing, and are more critical of, our on-line lectures than we have been for traditional lectures. We have adopted this mode for most aspects of our first year preclinical program. At this stage we have positive preliminary results and look forward to further feedback from our embedded evaluation of the program this year.

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Functional Role Of TRPC5 Channels In Aortic Baroreceptor

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Introduction. Aortic baroreceptor serves as mechanosensor that detects blood pressure in aortic arch. However, the molecular identity of the mechanosensor is not well understood. TRPC5 channel is a non-selective cation channel. It has been reported to be sensitive to hypo-osmolarity and pressure (1).
Aims. To investigate the functional role of TRPC5, a mechanosensitive ion channel, in aortic baroreceptor.
Methods. Immunohistochemistry and immunoblots, electrophysiological studies and Ca^{2+} imaging were performed to investigate the expression and function of TRPC5. Aortic depressor nerve activity was recorded in wild-type and $TRPC5^{-/-}$ mice.
Results. The expression of TRPC5 channels was found in the aortic baroreceptor nerve terminal, the nerve fiber and the ganglion region. In Ca^{2+} imaging studies of cultured aortic baroreceptor neurons, a TRPC5 potentiator daidzein (50 μmol/L) was able to potentiate the hypotonicity-induced $[Ca^{2+}]_I$ response while a TRPC5 blocking antibodies T5E3 (15 μg/ml) inhibited the response. Electrophysiological studies showed that hydrostatic pressure could activate whole-cell electrical current in cultured baroreceptor neurons and the current displayed a double rectifying *I-V* relationship, which is typical of TRPC5. Furthermore, *trpc5* knockout mice manifested a significant reduction in aortic depression nerve activity and baroreflex response upon blood pressure elevation when compared with wild-type mice. Knockout of TRPC5 also resulted in blood pressure instability in mice.
Discussion. Taken together, our study provides the evidence that TRPC5 is involved in pressure sensing of aortic baroreceptor neuron and participated in aortic baroreceptor function.

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Endothelium-Dependent Vasoconstrictor Signals Requiring Activation Of Soluble Guanylyl Cyclase In Isolated Arteries

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Introduction. Thymoquinone causes an endothelium-dependent augmentation of contraction in isolated arteries, similar to that evoked by hypoxia.
Aims. *Ex vivo* experiments were designed to study the mechanisms underlying this unexpected response.
Methods. Arterial rings with or without endothelium were suspended in organ chambers for isometric tension recording. Certain rings were incubated with inhibitors of nitric oxide synthase (L-NAME, 10^{-4} M), soluble guanylyl cyclase (sGC; ODQ, 10^{-5} M), rho-associated protein kinases (Y-27632, 10^{-5} M), L-type- (nifedipine, 10^{-5} M) or T-type voltage-gated calcium channels (ML-218, 10^{-4} M), while others were calcium-depleted (n=4-7). The rings were contracted with phenylephrine (10^{-6} M, rat aortae) or prostaglandin $F_{2\alpha}$ (10^{-7} – 10^{-5} M, porcine coronary arteries) and exposed to increasing concentrations of thymoquinone. Some rings were used to measure cyclic nucleotide level by HPLC-MS/MS (n=4-6).
Results. Thymoquinone caused a sustained further increase of tension in rings with endothelium, which was prevented by endothelium-removal, L-NAME and ODQ. Incubation with the NO-donor DETA NONOate (10^{-5} M) in L-NAME-treated rings restored and even increased the contractile response to thymoquinone, while treatment with 8-bromo cyclic GMP (10^{-4} M) or pyrophosphate (10^{-3} M) of ODQ-treated rings did not. HPLC-MS/MS measurements revealed that thymoquinone increased the production of cyclic IMP. Y-27632, nifedipine and calcium depletion inhibited the thymoquinone-induced contraction in porcine but not in rat arteries, while ML-218 reduced the phenomenon in rat but not in porcine arteries.
Discussion. The augmentation caused by thymoquinone requires endothelium-derived NO and activation of sGC, as described for hypoxia. In addition, both thymoquinone- and hypoxia-induced augmentation require production of cyclic IMP, altering intracellular calcium handling.
Thymoquinone can serve as a pharmacological tool to elicit endothelium-dependent vasoconstrictions that require activation of soluble guanylyl cyclase.

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Apolipoprotein A-I Restores Endothelial Function in Rats with Arthritis

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Introduction. Endothelial dysfunction is a key event in the development of atherosclerosis and has been identified in patients with rheumatoid arthritis and in rats with experimental arthritis. We have recently shown that apolipoprotein A-I (apoA-I), the most abundant apolipoprotein in high density lipoproteins (HDL), and reconstituted HDL [(A-I)rHDL] consisting of apoA-I complexed with phosphatidylcholine inhibit streptococcal cell wall peptidoglycan-polysaccharide (PG-PS)-induced arthritis in female Lewis rats.
Aim. This study asks if apoA-I also improves endothelial dysfunction in rats with arthritis.
Methods and Results. A single intraperitoneal injection of PG-PS (15 mg/kg) or an equivalent volume of saline (control) was administered to female Lewis rats. After four days the PG-PS-treated animals had acute joint inflammation, elevated circulating inflammatory cytokine levels, and had aortic endothelial dysfunction. Intravenous infusions of lipid-free apoA-I (8 mg/kg) 24 h pre- and 24 h post-PG-PS administration decreased the acute joint inflammation, reduced plasma TNF-α, IL-6 and IL-1β levels, and restored aortic endothelial function with an improvement in aortic vasorelaxation and an increase in guanosine 3',5'-cyclic monophosphate (cGMP) production at day 4 post-PG-PS injection. In *ex vivo* studies, incubation of aortic rings from control female Lewis rats with TNF-α (10 ng/mL) for 6 h impaired aortic vasorelaxation and decreased cGMP production. Pre-incubation of the aortic rings for 16 h with (A-I)rHDL (final apoA-I concentration 0.5 and 1.0 mg/mL) improved the TNF-α-induced impaired aortic vasorelaxation, and cGMP production. In addition, (A-I)rHDL induced endothelial nitric oxide synthase (eNOS) expression in human coronary artery endothelial cells (HCAECs) in a time- and dose-dependent manner. Incubation of HCAECs with TNF-α (1 ng/mL) for 6 h reduced HCAEC eNOS expression. Pre-incubation of the HCAECs for 16 h with (A-I)rHDL restored the TNF-α reduced HCAEC eNOS expression.
Discussion. These findings establish that apoA-I improves endothelial dysfunction in rats with arthritis by, at least partly, inhibiting inflammatory cytokine induced endothelial dysfunction.

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Contribution of COX-1 and COX-2 to endothelial cell eicosanoid release ex vivo and in vivo

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Introduction. Endothelial cells convert arachidonic acid to eicosanoid mediators, such as the cardioprotective hormone, prostacyclin. This occurs through biosynthetic enzyme pathways including cyclo-oxygenase (COX), the target of non-steroidal anti-inflammatory drugs. Although it is often said that endothelial cells primarily express the COX-2 isoform and that this explains the cardiovascular side effects of COX-2 inhibitors, we have previously shown COX-1 to be the major driver of prostacyclin release. The role of COX-1 vs. COX-2 in synthesis of other eicosanoids has not been addressed.
Aims. Here we have performed a full 'eicosanomic' analysis to determine the relative contribution of COX-1 and COX-2 to release of a full range of eicosanoid mediators by endothelial cells in vivo and ex vivo.
Methods. Experiments were performed on wildtype, COX-1$^{-/-}$ and COX-2$^{-/-}$ mice. Ex vivo, isolated aortic rings were stimulated with A23187 Ca^{2+} ionophore (50µmol/L in DMEM) for 30 mins. In vivo, under anaesthesia (isoflurane, 5% inhalation), mice were treated with bradykinin to active the endothelium (100nmol/kg in saline, intravenous) and after 5 mins blood collected from the carotid artery. Levels of 48 eicosanoids were measured in media/plasma using liquid chromatography-tandem mass spectrometry.
Results. Ex vivo, A23187-stimulated aortic rings released predominantly prostacyclin, 12-HETE/HEPE and PGE_2 with lower levels of 11- and 15-HETE/HEPE and PGD_2. Production of prostacyclin, PGE_2, PGD_2, 11- and 15-HETE/HEPE was abolished by COX-1 deletion but not by COX-2 deletion. 12-HETE/HEPE levels were not altered by deletion of either COX isoform. In vivo, 12-HETE/HEPE, PGE_2 and its major metabolite and prostacyclin were the most abundant eicosanoids with lower levels of 5-, 11- and 15-HETE/HEPE. Deletion of COX-1 but not COX-2 abolished production of prostacyclin, PGE_2 and its metabolite. Levels of 5-, 11-, 12- and 15-HETE/HEPE were not reduced by COX-1 or COX-2 deletion.
Discussion. These data firmly demonstrate that COX-1 and not COX-2 mediates synthesis of the full range of eicosanoid mediators by endothelial cells in vivo and ex vivo. They also highlight 11- and 15-HETE as novel endothelial COX-1 products.

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Rilmenidine Attenuates Ecstasy-induced Injury Of Cultured Murine Serotonin Neurones

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Introduction. Addiction and toxicity of ecstasy (3,4-methylenedioxymethamphtamine, MDMA) via actions on biogenic amine neurones are well documented. Oxidative stress, DNA damage and ubiquitinated inclusions contribute to MDMA-mediated neurotoxicity, but difficulties associated with primary culture of serotonin (5-HT) neurones have prevented delineation of its injury mechanisms in mammalian neurones.
Aims. To profile MDMA toxicity in primary cultured 5-HT neurones and to evaluate possible neuroprotective actions of rilmenidine.
Methods. Brain tissue containing rostral raphe nuclei (embryonic day 14-16 mice; mothers received inhalation anaesthesia, isoflurane) was digested, cells plated and maintained in microwell plates or on glass coverslips (0.1-0.2 x 10^6 cells/well, 12 days). Cellular and molecular techniques as described (1).
Results. Measurements of viability and [^{3}H]5-HT uptake at 24 h indicated cell death after oxidative stress (hydrogen peroxide) and autophagy (rapamycin) with dieback of 5-HT neurites. MDMA (0.1, 0.5 & 1 mmol/L, 24-72 h) produced time- and concentration-dependent reductions in cell viability and [^{3}H]5-HT uptake, and dieback of 5-HT neurones was slower than for hydrogen peroxide and rapamycin. Western immunoblotting for microtubule associated protein light chain 3 (LC-3) revealed autophagosome formation after treatment with MDMA and rapamycin. Confocal analyses after cytochemistry for 5-HT, Hoechst and LC-3 indicated MDMA induced autophagic activity as shown by abundant LC3-positive puncta within 5-HT neurones. Rilmenidine (1 µmol/L) protected against MDMA-induced injury (0.1 & 0.5 mmol/L) with preservation of 5-HT neuritic trees (>80%, image analysis) and reduction of LC-3 puncta.
Discussion. MDMA-induced injury appeared to involve autophagic programmed cell death and attenuation by rilmenidine suggests its potential use to prevent brain injury following MDMA abuse.

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Tolerance To the Respiratory Depressant And Antinociceptive Effects Of Morphine

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Introduction. Tolerance is known to develop to the antinociceptive effects of morphine (1) whereas the development of tolerance to the respiratory depressant effects of morphine has been questioned (2)
Aims. This investigation compared the development of tolerance to the respiratory depressant and antinociceptive effects of morphine and examined the ability of ethanol to reverse the tolerance observed.
Methods. Respiration was measured in adult male CD-1 mice as minute volume using whole body plethysmography. Antinociception was measured by tail flick latency to a thermal stimulus (52 ^{o}C). To induce morphine tolerance mice were implanted with a 75 mg morphine pellet (implanted s.c. under 3% isoflurane anaesthesia) for 6 days or given twice daily injections of morphine (20 mg/kg i.p.) for 5 days.
Results. Mice showed significant respiratory depression on day 1following morphine pellet implantation (152.4±4.1 ml/min pre-surgery vs 93.6±5.2 ml/min day 1 $p<0.05$; n = 8 but thereafter respiration returned to control levels by days 5-6. Significant antinociception was only observed on day 1 post-implantation (4.4±0.3 sec pre-surgery vs 13.1±1.7 sec day 1 $P<0.05$; n =8). By days 2-6 tail flick latency had returned to control levels. With twice daily morphine (20 mg/kg) injections over 5 days the respiratory depressant effect of morphine (10 mg/kg) did not decrease i.e. no tolerance developed. However, complete tolerance to the antinociceptive effects of morphine (10 mg/kg) was observed on day 3 of the twice daily injection protocol (4.8±0.2 sec twice daily saline vs 5.1±0.2sec twice daily morphine. $P>0.05$; n=6). As previously reported for antinociception tolerance (1), ethanol (0.3 g/kg i.p.) also reversed tolerance to the respiratory depressant effects of morphine in morphine pellet implanted mice.
Discussion. These data provide evidence that tolerance to morphine respiratory depression and antinociception develop at different rates. However tolerance to both morphine respiratory depression and antinociception are reversible by low doses of ethanol.

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The Effects Of Coffee Constituents On The Activity Of Indoleamine 2,3-Dioxygenase And Markers Of Inflammation In Differentiated THP-1 Cells

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Introduction. Depression is a highly prevalent disorder with a complex etiology. Current hypotheses implicating neuroinflammation and an alteration in tryptophan catabolism, predominantly through the kynurenine pathway and indoleamine 2,3-dioxygenase (IDO), better explain the pathophysiology of depression than older theories (1). Caffeinated coffee, one the most widely consumed beverages worldwide, has been shown to have antidepressant-like effects in epidemiological studies, an effect not shown with the consumption of decaffeinated coffee or caffeine alone (2, 3).
Aims. The aim of this study was to evaluate the effects of coffee constituents on tryptophan catabolism and inflammation in interferon-induced neuroinflammatory *in vitro* models.
Methods. Differentiated THP-1 cells were pre-treated with coffee constituents and then treated with interferon gamma for 24 hours. Supernatants were then collected and analysed for changes to the kynurenine to tryptophan ratio, using HPLC, and pro-inflammatory markers, using ELISA.
Results. Several key coffee constituents were shown to increase or decrease the kynurenine to tryptophan ratio, indicating IDO activity, and the production of the pro-inflammatory markers TNF-α, IL-6 and IL-1β.
Discussion. This study provides novel evidence for the role of biologically active coffee constituents in modulating IDO activity in a surrogate activated microglia model. This is likely to be due to their effects of the pro-inflammatory mediators TNF-α, IL-6 and IL-1β, which control IDO activity and are altered in the presence of coffee constituents in the model used. These findings provide an insight into the mechanisms of the antidepressant-like effects of coffee, as evidenced in epidemiological studies.

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Statins' Effects On LPS-Induced Neuroinflammation In Vivo

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Introduction. HMG-CoA reductase inhibitors (statins) are amongst the most commonly prescribed medications worldwide. While negative cognitive effects of statin treatment have been reported, many studies suggest chronic statin use may in fact be beneficial for cognition (1). As inflammatory markers have been associated with deficits in cognitive function, understanding statins' effects on neuroinflammation may clarify the mechanisms contributing to their cognitive effects.
Aims. This study looked to determine the effects of short term administration of atorvastatin, pravastatin, rosuvastatin, and simvastatin on an in vivo model of neuroinflammation.
Methods. Male C57BL/6J mice were given atorvastatin, pravastatin or simvastatin (10.4 mg/kg per day), rosuvastatin (5.2 mg/kg per day), or saline once daily for 3 days (n=7 per group). Lipopolysaccharide (LPS; *E. coli* 055:B5) 2 mg/kg was given via intraperitoneal injection 24 hours after final statin dose to induce neuroinflammation. The open field test (OFT), tail suspension test (TST) and forced swim test (FST) were conducted, and animals sacrificed 2 hours post-LPS. Whole brains and serum were collected; changes to a panel of 40 cytokines were screened via antibody array, and IL-1β and TNF-α levels quantified via enzyme immunoassay. Data were analysed via ANOVA & significance defined as $P<0.05$.
Results. LPS treatment significantly reduced locomotor activity in the OFT and increased immobility in the FST and TST compared to saline ($P<0.05$). Pravastatin and rosuvastatin significantly attenuated FST and TST immobility time, with rosuvastatin also improving OFT locomotor activity ($P<0.05$). Rosuvastatin, pravastatin and atorvastatin reduced LPS-induced TNF-α ($P<0.05$) however IL-1β release was unaffected by statins. Relative cytokine release varied between statins across the panel of 40 cytokines screened.
Discussion. Our results suggest statins have differing effects on neuroinflammation in vivo. Pravastatin and rosuvastatin decrease TNF-α release, which correlates with reduced anxious and depressive-like behaviours. Further studies are required to clarify the precise inflammatory pathways involved.
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Receptor Localisation Shapes The Spatiotemporal Dynamics of Mu-Opioid Receptor Signalling

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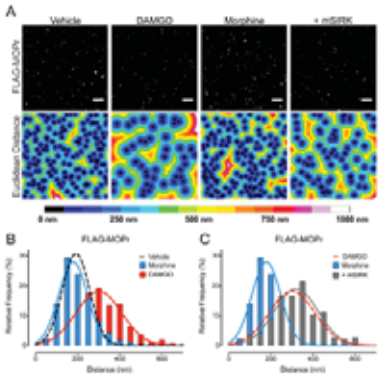

Introduction. Receptor signal compartmentalisation (the restriction of second messenger signalling in space and time) provides a mechanism whereby receptors can direct the assembly of focused "platforms" that facilitate second messenger production, the organisation and scaffolding of effectors, and co-ordination of regulatory events. This study is focused on the mu-opioid receptor (MOPr), the target of the most commonly used opioid analgesics. Morphine still remains the mainstay analgesic for the treatment of chronic pain, despite its use being still severely limited by undesirable on-target effects such as respiratory depression (potentially fatal), the development of tolerance and dependence, and gastrointestinal complications.
Aim. To investigate the spatial and temporal signalling profiles of the MOPr when activated by morphine or by a prototypical MOPr agonist (DAMGO) in model cell lines and in primary cultures of dorsal root ganglion (DRG) neurons.
Methods. We used BRET to measure endocytic trafficking and FRET biosensors to assess signalling from bulk compartments in single cells.
Results. We show that the morphine-activated MOPr displays a distinct spatiotemporal signalling profile compared to the DAMGO-activated receptor, and that these distinct profiles are associated with different plasma membrane localisation of MOPr induced by the two ligands.
Discussion. MOPr-mediated signalling is highly compartmentalised and that GPCR plasma membrane localization can lead to distinct spatiotemporal signalling in a ligand-dependent manner.

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RXFP1 and RXFP2, Peptide GPCRs With A Unique Tethered Ligand Transactivation Mechanism On Obligatory Dimers?

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The receptors for the peptide hormones relaxin and insulin-like peptide 3 (INSL3), RXFP1 and RXFP2, respectively, are members of the leucine-rich repeat (LRR) containing GPCR family (LGRs). This family includes the receptors for the glycoprotein hormones LH, FSH, TSH and the R-spondin receptors LGR4, LGR5 and LGR6 characterized by large ectodomains containing LRRs. RXFP1 and RXFP2 are unique in the LGR and GPCR family in that they contain an LDL class A (LDLa) module at their N-terminus. RXFP1 is of considerable therapeutic interest as relaxin has successfully completed a phase III clinical trial for the treatment of acute heart failure (AHF). Relaxin has an insulin-like two chain structure linked by 3 disulfide bonds, it is expensive to prepare, is not orally active and has a short in vivo half-life necessitating constant intravenous infusion. Hence although it is demonstrating clear efficacy with an exceptional safety profile as an AHF treatment, a small molecule RXFP1 agonist would have many advantages. However RXFP1 has a complex mechanism of ligand mediated activation that needs to be understood to enable the development of small molecules that exactly mimic the mode of relaxin mediated activation.
The extracellular domain (ECD) and transmembrane domain (TMD) of RXFP1 are both required for binding and activation. Relaxin binds with high affinity to the ECD, requiring both the LRR domain and the linker between the LRRs and the LDLa module, and with low affinity to the TMD. However binding alone is not sufficient for activation as receptor signalling is driven by a novel mechanism involving the LDLa module. We have demonstrated that the LDLa is essential for activation, but not binding, and seems to act like a tethered agonist and activate the TMD, potentially by transactivating a partner protomer in a dimeric complex. Mutation of specific LDLa residues does not influence ligand binding but leads to loss of function and biased signalling again highlighting that the LDLa is likely the true ligand of the receptor. This presentation will discuss this unique mechanism of GPCR activation which may involve ligand mediated transactivation of obligatory dimers by a tethered agonist LDLa module as well as the role of the LDLa module in ligand directed (biased) signalling.

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Molecular Pharmacology of Receptor Complexes

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Arginine vasopressin (AVP) and angiotensin II (AngII) play key roles in fluid homeostasis and blood pressure regulation, principally via activation of vasopressin receptor 2 (V_2) and angiotensin II receptor 1 (AT_1) respectively. Furthermore, there is increasing evidence for angiotensin II receptor 2 (AT_2) forming complexes with and modulating AT_1 (1). Mutations in the V_2 receptor can result in fluid homeostasis disorders (2-4), namely nephrogenic diabetes inspidus (NDI; loss-of-function) and nephrogenic syndrome of inappropriate antidiuresis (NSIAD; gain-of-function).
The aim was to profile AT_1-V_2 and AT_1-AT_2 heteromer complexes, as well as NDI- and NSIAD-causing mutations, in live cells in real-time. Bioluminescence resonance energy transfer (RET) was utilised predominantly, including in the GPCR-heteromer identification technology (GPCR-HIT) configuration (5), along with time-resolved fluorescence RET assays to measure cAMP and inositol-phosphate production (4). These molecular pharmacology approaches were complemented by in vivo studies of AT_1-V_2 functional interaction in the sub-total nephrectomy model of kidney disease, in a similar manner to our recently published study of AT_1-CCR2 heteromers (6).

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Investigating GPCR Dimerization and Complex Formation with Fluorescent Ligands

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Previous work, using fluorescent adenosine receptor agonists and antagonists, has provided novel insights into the allosteric regulation of adenosine A_3 (A_3AR) and A_1 (A_1AR) receptors by allosteric ligands and receptor dimerization in single living cells (1-2). We have also used a fluorescent analogue of CGP12177 to investigate ligand binding to the human β1-adrenoceptor. This work has demonstrated that there is negative cooperativity between the two different ligand-binding conformations of the β1-adrenoceptor activated by catecholamines and CGP12177 respectively (3). Finally, we have used fluorescence correlation spectroscopy (FCS) to investigate ligand binding to A_1AR and A_3AR in small 0.2 μm^2 microdomains of single living cells (4). FCS studies with a fluorescent A_3-agonist have enabled high affinity labeling of the active conformation (R*) of the receptor (4). We have also used a fluorescent adenosine A_3-antagonist (CA200645) to study the binding characteristics of antagonist-occupied receptor conformations (R) in membrane microdomains of single cells (5). FCS analysis of CA200645-occupied A_3ARs revealed two species that diffused at 2.29 μm^2/s and 0.09 μm^2/s, respectively. FCS analysis of a GFP-tagged A_3AR exhibited a single diffusing species (0.09 μm^2/s) that was not altered by pre-treatment with A_3-agonists or A_3-antagonists. The binding of CA200645 to the A_3AR was antagonized by nanomolar concentrations of the A_3-antagonist MRS 1220, but not by NECA (up to 300nM) consistent with labeling of the inactive conformation (R) of the receptor. Investigation of the dissociation kinetics of CA200645 provided further support for allosteric regulation of this receptor by homodimerization (5).

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Synergistic Anticancer Effects Via Beneficial Interactions Between Chinese Medicinal Herb and Anti-Cancer Drugs

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Medicinal herbs are often concurrently administered with orthodox drugs for the treatment of varieties of diseases worldwide to produce beneficial outcomes via PK and/or PD herb-drug interactions. However, because of the unique nature of medicinal herbs, which have multiple ingredients to interact with multiple targets leading to different bioactivities, the investigation of herb-drug interactions and their mechanisms underlying such interactions are challenging and very difficult. To date, the scientific investigation with sound evidences to support the beneficial clinical outcomes of such combinational usefulness are far from satisfactory. In the recent years, our research team has been working on herb-drug interactions using our established integrative PK/PD multidisciplinary approach. In this presentation, our integrative approach to investigate beneficial herb-drug interactions between traditional Chinese medicinal herbs and some anticancer drugs and to delineate the mechanisms underlying such beneficial interactions will be addressed. Using one Chinese medicinal herb as an example, our findings including 1) the identification of true herbal ingredients, which do not directly produce any pharmacological actions, but rather serve as “pro-bioactive” components to elicit their bioactivities after biotransformation in the body; and 2) the delineation of the mechanism of synergistic anticancer effects due to the different herbal ingredients interacting with differential targets and their functions as the “assistants” to facilitate the effects of the anticancer drug leading to the beneficial action will be illustrated.

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Contamination, Adulteration And Side Effects Of Chinese And Herbal Medicines

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Herbal medicine is increasingly popular due to public perceptions of safety. Despite these perceptions, there are health risks from herbal medicines. A survey of the Australian Adverse Drug Reactions reporting system revealed significant adverse effects. Adverse reactions can be due to the intended ingredients or adulteration or contamination. Adulteration and contamination of herbal and complementary medicines continues to be a significant problem worldwide. Herbal medicines may contain conventional medications, heavy metals and undeclared plant or animal material. One of the challenges of assessing herbal medicines for these materials is the sheer complexity of herbal preparations themselves, and the range of potential contaminants. Identifying herbal materials is often confounded by the lack of biomarkers and that processing removes morphological markers that identify herbs. Next generation DNA sequencing (NGS) can be used to rapidly evaluate complex mixtures unable to be tested effectively by previous techniques. We used a combination of NGS and toxicological screening to evaluate contamination and adulteration in Traditional Chinese Medicines. Twenty-six pre-packaged TCM samples (capsules, tablets, and herbal teas) were purchased from retail stores and TCM practitioners in Adelaide, South Australia. 17 (65%) had undeclared content found by DNA and/or toxicological analyses. In some herbals DNA detection correlated with toxicological data (eg Ephedrine and *Ephedra* species). This study shows that combined DNA and toxicological screening enables more effective evaluation of TCMS.

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Astragalus Saponins: The Potential Benefits and Prospective Role in the Protection Against Gastrointestinal Cancers

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The potential of total *Astragalus* saponins extract (AST) in treating gastrointestinal cancers had been investigated. We have demonstrated that AST caused significant growth inhibition and pro-apoptotic effects in human colon adenocarcinoma cells (50-80 μg/ml) and tumor xenograft (100-200 mg/kg, i.p.) in nude mice, with synergistic antitumor activity with the conventional chemotherapeutic drug combo 5-FU/oxaliplatin or vinblastine and alleviation of the associated drug-induced toxicity (1,2). Its universal chemotherapeutic property had been exhibited in 8 different human cancer cell lines. We have identified NSAID-activated gene (NAG-1) as a molecular target of AST, which could be correlated with modulation of the upstream PI3K-AST signaling pathway (3). AST also exhibited promising anti-carcinogenic effects in hepatocarcinoma cells via an ERK-independent NF-κB signaling pathway. Nevertheless, the invasiveness of AGS gastric adenocarcinoma cells through the Matrigel membrane in a Cell Invasion Assay System was significantly reduced upon AST treatment, with concomitant downregulation of pro-angiogenic factors such as VEGF as well as the metastatic mediators MMP2 and MMP9. The gene profile of HCT 116 colon adenocarcinoma cells following AST treatment from a customized cDNA array further confirms this. In a modified liver metastasis model, the size of the tumor mass has been reduced by more than 85% in AST-treated animals. Glucose-regulated proteins (GRP) are induced to promote tumor survival, metastasis and drug resistance. Our most recent findings exemplify that calpains have played a permissive role in the modulation of GRP78 and consequent regulation of ER stress-induced apoptosis caused by AST in the cancer microenvironment. Data obtained from these studies could facilitate future establishment of AST as an effective target-specific chemotherapeutic and adjuvant agent, with known signaling pathways and unique molecular targets.

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Heavy Metals in Chinese and Herbal Medicines

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Herbal materials and herbal medicines are widely used as complementary and alternative medicines or natural products in many countries. These products contain biologically essential and non-essential heavy metals and metalloids. Although many herbal products are safe to use, there are increasing concerns of the potential risks involved in herbal products containing high levels of heavy metals, such as lead, arsenic, cadmium and mercury. It has been shown that the exposure to herbal products with high levels of heavy metals can lead to adverse events and poisoning. Traditionally, some heavy metals are used as therapeutic agents in Chinese and Ayurvedic medicines. In many other cases, heavy metals are introduced into products as contaminants from production procedures including cultivation and processing. Various surveys have found a number of commonly used herbal materials and herbal medicines contain high levels of heavy metals. The value of added heavy metals in traditional herbal medicines is highly debatable though there is evidence on variations in species and toxic profile of heavy metals in traditional medicines. Assessing the potential risks of heavy metals in herbal products should be not based on the amounts of metals in the products alone, but also consider the source, distribution, bioavailability and bioaccessibility of metals that may be of concern. Currently there remains a lack of international accepted quality control standards for herbal medicines, including consideration of the content of heavy metals in these products. A coordinated international approach is necessary to develop such standards to ensure the safe use of herbal medicines by the general public, and also promote the evidence based practice of herbal medicines.

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IUPHAR: Mission for Pharmacology

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While new medications are desperately needed for the treatment of conditions from infectious disease, to cancer and neuropsychiatric illness, the role played by pharmacology in addressing this issue is under-appreciated by many. The mission of IUPHAR is to change this perception within both the clinical and basic research communities. This is accomplished by fostering education and disseminating information worldwide. The successful IOSP initiative is one example of educational outreach. Moreover, IUPHAR has formed partnerships with ASPET to create an educational web site and with the BPS to establish the Guide to Pharmacology (guidetopharmacology.org). The latter combines information from the IUPHAR receptor database (NC-IUPHAR) with the BPS Guide to Receptors and Channels (GRAC) compendium. The NC-IUPHAR has grown from a committee formed to classify G-protein-coupled receptors to an organization with >80 expert subcommittees (672 scientists) working together to create a database on pharmacological targets in general. Through these efforts, and the various initiatives undertaken by IUPHAR sections and the Clinical Pharmacology Division, there is a growing appreciation for the field among organizations and individuals engaged in drug discovery and development, which helps ensure that the discipline continues to flourish. We are very actively searching innovative ways to finance its long term future. Nevertheless, drug discovery, although a great hope for the future, has not lead to all the benefit that we expected. Indeed the failure rate in the development of drugs for Alzheimers is 99.6%. This is unsustainable. One reason may be that other targets are more important than those addressed. Hence we have recommendations on alternative splicing, heterodimers, allostery, epigenetic targets and work on non-coding RNAs, in alliance with HGNC. This conference shows work involved in the aging process. The immunopharmacology section has started a major initiative in immunological/inflammatory drug targets. We also support the role of academic drug discovery in rare and tropical diseases.

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Ethnic Diversity: Implications for Drug Response and Drug Development

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Inter-ethnic differences in responses to medicines have been investigated systematically since the early clinical studies of drug effects. It is now well understood that a range of intrinsic and extrinsic ethnic factors can potentially contribute to inter-ethnic differences in pharmacokinetics and pharmacodynamics and therefore drug efficacy or safety (target and non-target mediated). For a limited number of drugs ethnic group specific dosage recommendations have been approved. The drug doses approved can also vary across countries, potentially reflecting separate drug development strategies rather than inter-ethnic differences in the dose-response relationship. Currently multiregional clinical trials are the cornerstone of new drug development and collect evidence of the efficacy and safety of new compounds in patients from a diverse range of ethnic groups. This is an opportunity to obtain clinical data in specific ethnic groups as well as in specific local populations. However, the potential for inter-ethnic differences in drug response also adds complexity. The clinical trial results in a subgroup of patients in a multiregional clinical trial can be influenced by a range of factors, including chance. Ethnic sensitivity assessment can inform both the planning of multiregional drug development programmes and the interpretation of clinical trial results. Early knowledge of the determinants of the pharmacokinetics and pharmacodynamics of a new drug in development and the profile of these determinants across ethnic groups is consequently important. When inter-ethnic differences in response could potentially occur, targeted clinical pharmacology studies in specific ethnic groups can provide valuable insights which are pivotal to the planning of subsequent global clinical development strategies.

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Evaluation Of Beta-2-Microglobulin And The Framingham Risk Score In Mortality Prediction

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Introduction. We previously showed that beta-2-microglobulin (B2M) is associated with cardiovascular mortality (1). However, its clinical relevancy is not understood.
Aims. This study sought to evaluate the relationship between B2M and the Framingham Risk Score (FRS) in predicting mortality.
Methods. 6,554 adult participants of the Third National Health and Nutrition Examination Survey were included in the analysis. Serum B2M level was used in multivariable Cox regression analysis to predict all-cause and cardiovascular mortality. 10-year FRS of coronary heart disease was used for analysis. Reclassification of mortality was assessed using integrative discrimination index (IDI) and category-less net reclassification improvement (NRI).
Results. During a median follow-up of 13.5 years (79,528 person-years), 2,524 and 1,150 participants died from all causes and cardiovascular causes, respectively. Serum B2M level was linearly associated with FRS ($P<0.001$). After having adjusted for FRS, quartile 4 of B2M was significantly associated with all-cause (hazard ratio (HR)=5.53, 95% CI: 3.45-8.85) and cardiovascular mortality (HR=5.21, 95% CI: 2.18-12.49). Similar results were obtained when B2M was analysed as a continuous variable. Serum B2M, when added to FRS, showed significant improvement in reclassification in terms of IDI and category-less NRI.
Conclusion. Serum B2M level is an independent predictor of cardiovascular and all-cause mortality in the general population, and adds to FRS in assessing mortality risk.

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The Impact Of Chronic Perindopril Therapy On The Onset Of The Metabolic Syndrome And Obesity In Aged High-Fat High-Carbohydrate (HFHC) Fed WKY Rats

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Introduction. Obesity, insulin resistance, hypertension, oxidative stress and inflammation that lead to cardiovascular remodelling are heavily implicated in the pathophysiology of both the metabolic syndrome and hypertension. While novel natural compounds may hold promise as potential cardioprotective therapies, well established antihypertensive medications such as perindopril (P) may also possess therapeutic properties that extend beyond lowering blood pressure (1), by preventing the onset of obesity.
Aims. To reverse the onset of obesity, hypertension and associated secondary cardiovascular complications in aged HFHC fed normotensive WKY rats.
Methods. At 16wks of age WKYHFHC rats began HFHC feeding for 20 weeks. Perindopril therapy started at 24wks of age for 12wks (oral gavage 1mg/kg/day) with all rats sacrificed at 36wks of age with biometric, biochemical, isolated vessel reactivity and cardiac electrophysiological experiments conducted. AEC approval A12/04-281; (n=12 for all groups; *p<0.05 vs. WKY; **p<0.05 vs. WKYHFHC)
Results. HFHC feeding increased bodyweight (WKY 365±8; WKYHFHC 398±7*g), fat pad deposition (WKY 15±1; WKYHFHC 31±1*mg/g bwt), and blood pressure (WKY 140±4; WKYHFHC 162±2*mmHg) accompanied by a loss of endothelial vascular response and a prolonged ventricular action potential. ACE inhibition reversed and normalised bodyweight (WKYHFHC+P 371±8**g), fat pad deposition (WKYHFHC+P 13±1**mg/g bwt), hypertension (WKYHFHC+P 132±3**mmHg) vascular dysfunction and cardiac action potential duration.
Discussion. Perindopril reversed and normalised all aspects of the metabolic syndrome and secondary cardiovascular dysfunction in HFHC fed WKY rats. This novel finding suggests the RAAS may be involved in potentiating weight gain and adiposity during the onset of obesity.

(1) Ceconi, C. et al. (2009). Results from the PERTINENT study." Atherosclerosis **204** (1): 273-275.

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Effects Of Fructose Feeding On Cardiovascular Function And Body Composition In GPR55-/- Mice

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Introduction. Metabolic syndrome is associated with increased cardiovascular risk. GPR55 expression is increased in obese individuals [1] and GPR55 activation increases glucose tolerance and plasma insulin levels suggesting a role for GPR55 in metabolic syndrome.
Aims. To determine the effect of fructose feeding on body composition, myocardial ischaemia/reperfusion injury and vascular function wild type (WT) and GPR55 knockout mice ($GPR55^{-/-}$).
Methods. $GPR55^{-/-}$ and WT (C57 BL/6J) mice (2 months old) were fed either normal drinking water (control; n=10 per strain) or 30% fructose water (fructose; n=10 per strain) for 12 weeks. Body composition was determined by Echo scanning at the end of the intervention period. Following anaesthesia with ketamine/xylazine (120mg/kg & 16mg/kg i.p.,) the hearts were removed, perfused in Langendorff mode and subjected to global myocardial ischaemia/reperfusion (30 min + 30 min) and infarct size measured using planimetry. Vascular function of carotid arteries was assessed by myography,
Results. In the controls, $GPR55^{-/-}$ mice exhibited a significantly higher fat mass (6.8±0.2g) compared to WT mice (4.2±0.1g; P<0.05), despite no difference in body weight. Fructose feeding induced a significant increase in fat mass in the WT mice (8.1±0.1g; P<0.05) but not in $GPR55^{-/-}$ mice. Control WT and $GPR55^{-/-}$ hearts developed similar sized infarcts (34±2% and 31±3% of LV), while fructose WT hearts had smaller infarcts (24±5%; P<0.05); this was not observed in the $GPR55^{-/-}$ group. Fructose induced a significant attenuation of the relaxant responses induced by methacholine and SNP in both strains compared to controls (P<0.01), which was greater in the $GPR55^{-/-}$ mice compared to the WT.
Discussion. These studies demonstrate that $GPR55^{-/-}$ mice exhibit a metabolic syndrome-type phenotype in terms of body composition and that fructose feeding induces marked vascular dysfunction that is exacerbated in the absence of GPR55.

(1) Moreno-Navarrete JM et al (2012). Diabetes **61**:281-291.

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Enhanced Serelaxin Signalling in Co-cultures of Human Primary Endothelial and Smooth Muscle Cells Mohsin Sarwar[1], Chrishan S Samuel[2], Ross AD Bathgate[3] & Roger J Summers[1], Monash Institute of Pharmaceutical Sciences[1] & Florey Neurosciences Institute[3], Parkville, Vic 3052, Australia.

Introduction. In the PhIII clinical trial, RELAX-AHF, 48 hr infusion of serelaxin (recombinant H2 relaxin), caused marked vasodilation in patients with acute heart failure (Ponikowski et al. 2013). However, the cellular mechanism(s) associated with its vascular effects in humans are poorly understood.
Aims. This study examined the effects of serelaxin in co-cultures of human primary endothelial cells (ECs) and smooth muscle cells (SMCs) on cAMP and cGMP signalling, markers of vascular function.
Methods. A co-culture model utilised cell culture inserts (Thincerts) to examine endothelium-dependent signalling. α-Screen cAMP and cGMP assays were conducted to examine serelaxin signalling.
Results. Stimulation of human umbilical vein endothelial cells (HUVECs) and human coronary artery endothelial cells (HCAECs) with serelaxin concentration-dependently increased cGMP accumulation in co-cultured HUASMCs and HUVSMCs to levels 2-2.5 fold higher than in monocultures. Pre-incubation of HUVECs and HCAECs with a NO-synthase inhibitor, L-NOARG (30µM, 30min), inhibited serelaxin-mediated (30nM) cGMP accumulation in HUVECs and HCAECs, but also inhibited cGMP accumulation in HUASMCs and HUVSMCs. Additionally in HCAECs but not HUVECs, pre-incubation with indomethacin (30µM, 30min), inhibited cGMP accumulation in HUASMCs and HUVSMCs. Serelaxin stimulation of HCAECs but not HUVECs also increased cAMP accumulation concentration-dependently in HUASMCs and HUVSMCs. Pre-incubation of HCAECs with indomethacin (30µM, 30min) but not L-NOARG (30µM, 30min) abolished cAMP accumulation in vascular SMCs.
Discussion. Serelaxin caused EC-dependent cGMP accumulation in vascular SMCs but also when added to HCAECs caused EC-dependent cAMP accumulation. The responses involved nitric oxide and GC activation in vascular SMCs but also in HCAECs, EC-derived prostanoid production. Thus serelaxin utilizes several mechanisms to modulate vascular tone in different vascular beds.

(1) Ponikowski, P. et al., 2013 European Heart Journal, 35(7), pp.431–441.

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Toxic Epidermal Necrolysis: Clinical Profile, Aetiology And Treatment In A Tertiary Hospital Over A 16-year Period
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Introduction. Toxic epidermal necrolysis (TEN) represents the most severe end of the spectrum in adverse drug reactions which carries a high mortality rate. TEN is rare and patients are mostly managed in tertiary referral centres.
Aims. This study aimed to describe the clinical profile, aetiological agents, treatment and outcome of TEN in a tertiary hospital in Hong Kong over a 16-year period.
Methods. A retrospective analysis was conducted. Patients admitted to Queen Mary Hospital, Hong Kong from 1 January 1999 to 31 December 2014 with a diagnosis of TEN were identified from the electronic medical database. Clinical data were retrieved from the database and medical records for analysis. Comparison between survivors and deceased were performed to identify any risk factors for mortality.
Results. 40 patients were identified from the database and the diagnosis of TEN was validated in 33 patients. Age ranged from 1 to 87 years (mean 52.5 years) and 19 (57.6%) cases were female. Anti-convulsants (28.1%) were the commonest implicated drugs, followed by allopurinol (15.6%) and antibiotics (12.5%). Intravenous immunoglobulin (IVIG) was administered in 16 patients (48.5%) while only one case was treated with systemic steroid. Case fatality rate was 25.0%. Acute kidney injury was strongly associated with mortality (hazard ratio: 15.35, P=0.004).
Discussion. TEN is rare in Hong Kong and is most likely caused by anticonvulsants, allopurinol or antibiotics. The mortality is considerable, especially when acute kidney injury has occurred.

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Serum Calcium and Incident Diabetes: A Retrospective Study in Hong Kong Chinese and Meta-Analysis

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Introduction. Bone and mineral metabolism plays a role in glucose metabolism. Recent studies suggested that serum calcium is also associated with the risk of diabetes.
Aims. We aimed to evaluate the association of serum calcium with incident diabetes in Hong Kong Chinese.
Methods. We conducted a retrospective cohort study on 1702 male and 4394 female Hong Kong Chinese aged 20 or above free of diabetes at baseline, and the incident diabetes was determined from electronic patient record databases. We also conducted a systematic literature review for similar studies and calculated an overall relative risk (RR) using a fixed-effect model.
Results. In 59130.9 person-years of follow-up, 631 participants developed diabetes. Serum total calcium (third quartile, HR 1.42; 95% CI 1.12-1.8; highest quartile, HR 1.41; 95% CI 1.11-1.79; as compared to the lowest quartile) were significantly associated with incident diabetes. Significant interactions with BMI and age were observed. Greater total calcium intake was significantly associated with less incident diabetes (comparing extreme quartiles, hazard ratio 0.78; 95% confidence interval 0.61-0.98). In meta-analysis of three studies including ours, with a total of 34,117 participants, the pooled RR of incident diabetes was 1.43 (95% CI 1.20, 1.70) in individuals with serum calcium level greater than 2.44 mmol/L.
Conclusion. Elevated serum total calcium and lower total calcium intake were associated with incident diabetes. The mechanism warrants further investigation.

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The Role Of Nationally Agreed Prescribing Indicators (NPIs) In Promoting Prudent Prescribing; Experience In Wales (2002-2014)

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Introduction. Prescribing should aim for optimal efficacy, safety and efficiency.
Aims. We used national indicators (NPIs) to measure prescribing standards in primary care in Wales, for benchmarking purposes, to help target appropriate interventions and to measure any subsequent improvement (1).
Methods. NPIs were agreed by national consensus using different measures of prescribing volumes (e.g. expressed per prescribing unit [PU] or defined daily dose [DDD]). They were endorsed by the All Wales Medicines Strategy Group and reviewed annually for continuing relevance, and were based on evidence-based clinical pharmacological principles.

NPIs	2002/3	2007/8	2013/4
Generic medicines (% of all prescriptions)	74	84	83
Antibiotics (items/1,000 PUs)	150	148	153
Hypnotics & anxiolytics (DDDs/1,000 patients)	3178	2479	1736
NSAIDS (DDDs/ 1,000 PUs)	2896	2565	2125
Ibuprofen & naproxen (% of all NSAIDs)	27	36	78

Results. Twenty-eight different NPIs were used over the period of study, although more than half of these were subsequent refinements of the original NPIs. Almost all have moved in the desired direction towards previously agreed targets, although antibiotic prescribing volumes are still particularly resistant to change in Wales (see table).
Discussion. NPIs are an explicit mechanism for applying clinical pharmacological principles to prescribing. Further work using NPIs is needed to examine the relative roles of education, peer comparison/ competition and incentivisation in achieving prudent prescribing in primary care.

(1) http://www.senedd.assembly.wales/documents/s10974/Action%20Point%20-%20Invest2save3%20English.pdf (accessed 30/01/2015) (224 words)

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Health Technology Appraisal And Access To Medicines; Experience Of The All Wales Medicines Strategy Group, 2002-2014

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Introduction. The All Wales Medicines Group (AWMSG) was established in 2002 to advise Welsh Government on the introduction of certain medicines into Wales.
Aims. We decided to analyse the acceptance rate of all medicines submitted by manufacturers to AWMSG for health technology appraisal (HTA) in NHS Wales, as well as separately for a sub-group of those medicines indicated for the treatment of conditions with a prevalence in the European Union of ≤ 5 in 10,000 (sometimes called “orphan medicines”).
Methods. HTA was conducted by AWMSG in its monthly meetings in public. “Recommended Medicines” were approved either according to the full licensed indication or for a restricted (“optimised”) indication.
Results. In the period September 1st 2002 to December 31st 2014, 184 (81%) of the 228 HTA’s resulted in the medicine being recommended, either in full or with restrictions (see Figure). In contrast only 20 (59%) of the 34 orphan medicines were recommended either in full or restricted (Fisher’s exact test, two-tailed p = 0.0072 c/w non-orphan medicines). In the case of ultra-orphan medicines (a sub-set of orphan medicines generally defined as being used to treat conditions with a prevalence of ≤ 1 in 50,000 in the EU), 8 (73%) of 11 medicines were recommended in full or with restrictions (Fisher’s exact test, two-tailed p = 0.2948, NS c/w other orphan medicines).
Discussion. Further work is required to develop timely, robust and transparent approaches to ensure that clinically effective medicines (including orphan and ultra-orphan medicines) can be made optimally available to patients within a finite health budget.

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Glucose Uptake in Response to activation of α_{1A}-Adrenoceptors Involves mTORC2, AMPK, and Rac1 Masaaki Sato[1,2], Anna L. Sandstrom[2], Linzi Lim[1], Dana S. Hutchinson[1], Saori Mukaida, Bronwyn A. Evans[1], Tore Bengtsson[2] and Roger J. Summers[1]. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University[1], Parkville, AUSTRALIA; Department of Molecular Bioscience, The Wenner-Gren Institute, Stockholm University[2], Stockholm, SWEDEN

Introduction. Stimulation of α_{1A}-adrenoceptors (α_{1A}-AR) causes glucose uptake in skeletal muscle involving phospholipase C and glucose transporter 4 (GLUT4) translocation to the plasma membrane (Hsu et al., 2004; Hutchinson et al., 2005). However the signalling pathways downstream of α_{1A}-AR activation linking to GLUT4 translocation remain to be identified.
Aims. To evaluate the signalling pathways activated by the α_{1A}-AR that are coupled to glucose uptake.
Methods. Signalling pathways were investigated using selective kinase inhibitors, siRNA and detected by measuring ^{3}H 2-deoxy-D-glucose uptake, western blots, α-screen assays and confocal microscopy.
Results. Noradrenaline (NA), and the selective α_{1A}-AR agonists A61603 and oxymetazoline, and the Ca^{2+} ionophore A23187 concentration-dependently increased glucose uptake in CHO-K1 cells stably expressing the α_{1A}-AR and GLUT4. The AMPK inhibitor Cmpd C or transfection of rictor but not raptor siRNA inhibited α_{1A}-AR-mediated glucose uptake. The Rac1 inhibitor NSC23766 inhibited glucose uptake to NA, A61603, oxymetazoline, and A23187 by 85%, 80%, 71%, and 81% respectively. Western blot and α-screen assays showed that α_{1A}-AR agonists had no effect on Akt and S6K phosphorylation but increased mTOR and AMPK phosphorylation. Confocal analysis showed increased GLUT4 translocation following stimulation by NA, A61603, oxymetazoline, and A23187.
Discussion. α_{1A}-ARs activate mTORC2, AMPK and Rac1 that have key roles in glucose uptake involving actin reorganization to cause translocation of glucose transporters to the plasma membrane.

(1) Hsu JH et al (2004) Planta Med. 70(12):1230-3
(2) Hutchinson DS et al (2005) Endocrinology 146(2):901-12

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Activation of Phospholipase Cβ By Non-dissociable Gq Proteins

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Introduction. The classical model of G protein activation entails the physical dissociation of Gβγ from the GTP-bound Gα subunit, thereby producing two separate signals for effector regulation. However, subunit dissociation does not seem to be required for Gs signalling in living cells (1). This raises the provocative possibility that some non-dissociated heterotrimers can in fact regulate downstream effectors.
Aims. Determine if subunit dissociation of Gq is required for the activation of phospholipase Cβ (PLCβ).
Methods. Gβ-Gαq fusion proteins with different linkers were constructed based on a previous study (2). Wild-type and constitutively active fusion proteins were examined for IP_3 formation in transfected cells.
Results. A Gβ-Gαq fusion protein with only one amino acid linking the two subunits and an activating mutation (β-1-αqQL) constitutively stimulated PLCβ in transfected HEK293 cells. Wild-type β-1-αqz5 which has been engineered detect Gi-coupled receptors (dopamine D_2, α_2-adrenergic, and adenosine A_1) efficiently mediated agonist-induced Ca^{2+} mobilization in a FLIPR assay. Molecular modelling predicts that PLCβ could associate with Gα and Gβγ simultaneously.
Discussion. The ability of β-1-αqQL to effectively activate PLCβ suggests that physical dissociation of Gq subunits is not an absolute requirement for Gq signalling in a cellular environment. Re-orientation of Gαq and Gβγ with respect to each other may be sufficient to allow productive interaction with PLCβ. Without the need for physical dissociation and subsequent re-association, the Gq/PLCβ pathway would be able to operate more efficiently. As PLCβ is known to interact with both Gαq and Gβγ, it remains to be determined if PLCβ can indeed form a fully functional complex with heterotrimeric Gq.

(1) Digby Y et al (2006) Proc Natl Acad Sci USA 103:17789-17794
(2) Klein S et al (2000) Proc Natl Acad Sci USA 97:3219-322

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Quantifying The Ligand Binding Affinity Of Vascular Endothelial Growth Factor (VEGF) Isoforms At VEGF Receptor 2 (VEGFR2) In HEK 293 Cells

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Introduction. The vascular endothelial growth factor receptor (VEGFR2) is critical for angiogenesis, proliferation and cell survival. Alternative splicing of the VEGF gene results in multiple isoforms of the VEGF ligand which have distinct physiological roles (1). However, quantitative analysis of the binding affinities of these ligands at the VEGFR2 is currently lacking.
Aims. To characterise the binding affinities of VEGF isoforms at the VEGFR2.
Methods. HEK293 cells stably expressing VEGFR2 tagged with a novel N terminal luciferase NLuc® (Promega) were seeded at 40,000 cells per well in 96 well plates. Bioluminescence resonance energy transfer (BRET) was measured between NLuc-VEGFR2 and fluorescently labelled $VEGF_{165}$a-TAMRA (1hr; 37°C). Competition binding experiments (with unlabeled VEGF isoforms) were conducted in the presence of a fixed concentration of $VEGF_{165}$a-TAMRA. The NLuc substrate furimazine was added 5 min prior to plates being read on a Pherastar plate reader (BMG). Ligand binding affinities were calculated assuming competition using K_d values for $VEGF_{165}$a-TAMRA from one site saturation analysis.
Results. Ligand binding affinities at VEGFR2 gave a rank order of $VEGF_{165}$a > $VEGF_{121}$a is greater than or equal to $VEGF_{165}$b > $VEGF_{189}$a > $VEGF_{145}$a (see Table 1).
Discussion. This work has, for the first time, quantified the ligand binding affinities of VEGF ligand isoforms at the VEGFR2.
(1) Woolard et al (2009)., Microcirculation (7), 572-92

Table 1: Binding affinities of VEGF ligand isoforms

Ligand isoform	pK_i (± S.E.M)	n
$VEGF_{165}$a	9.9 ± 0.1	5
$VEGF_{165}$b	9.4 ± 0.1	5
$VEGF_{189}$a	9.0 ± 0.1	5
$VEGF_{145}$a	8.2 ± 0.1	3
$VEGF_{121}$a	9.5 ± 0.1	5

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Reactive oxygen species and calcium signalling in the induction of hypoxia-mediated epithelial-mesenchymal transition

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Introduction. Hypoxia is a hallmark of the cancer microenvironment and induces epithelial-mesenchymal transition (EMT), a process whereby epithelial cells acquire more migratory and invasive characteristics. Hypoxia can be associated with altered calcium (Ca^{2+}) signalling through the production of reactive oxygen species (ROS), however, this association has not been fully characterised in breast cancer cells.
Aims. To assess the remodelling of Ca^{2+} signaling and ROS production in a hypoxia model of EMT in MDA-MB-468 breast cancer cells and to define the role of ROS and specific Ca^{2+}-related proteins in EMT induction in this model.
Methods. To induce EMT, MDA-MB-468 breast cancer cells were incubated in 1% O_2. Levels of ROS were assessed using the cell-permeable fluorogenic probe 2´,7´-dichlorodihydrofluorescin diacetate (DCFH-DA). Quantitative RT-PCR or immunoblotting were used to assess mRNA or protein levels of EMT markers and Ca^{2+}-related proteins. Dharmacon ON-TARGET*plus* SMARTpool siRNA was used to silence specific Ca^{2+} channels.
Results. Hypoxia increased the mRNA (24 h) and/or protein levels (48 h) of the EMT markers vimentin, N-cadherin, snail, twist and increased intracellular ROS levels (12 h). Among the fifty Ca^{2+}-related proteins assessed, four underwent significant mRNA up-regulation (3 to 10-fold) with hypoxia. Chelation of ROS with 10 mM N-acetylcysteine (NAC) significantly altered the expression pattern of these Ca^{2+}-related proteins and some EMT markers (N-cadherin). Silencing of TRPC1 channel, one of the four up-regulated Ca^{2+}-related proteins, significantly decreased hypoxia-induced vimentin protein expression.
Discussion. These results implicate an important role for ROS and Ca^{2+} signalling in the induction of hypoxia-mediated EMT in breast cancer cells.

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The Ins And Outs Of TLR4 Signalling In Sensory Ganglia

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Toll-like receptor-4 (TLR4) is classically associated with cells of the innate immune system to detect and respond to pathogens and tissue damage-associated molecules. Therefore, its presence on primary sensory neurones should not be a surprise given that they too are involved in detecting danger signals. But, why is TLR4 rarely expressed on the satellite glial cells which surround the soma of sensory neurones, and which are reported to possess immune-like cell properties? By studying primary cultures of dorsal root ganglion (DRG) cells isolated from adult rats, we clearly detected TLR4 mRNA in both mixed DRG cell cultures (neurones and glial cells) as well as in pure DRG glial cell cultures (satellite glial cells and Schwann cells) (1). TLR4-ir was detected on the surface of DRG neurones, but was only ever detected on the surface of glial cells in the absence of neurones (2). By examining the effect of DRG neurone-conditioned medium on TLR4-ir expression by DRG glial cells, we concluded that direct cell-cell contact was required for neurones to exert this inhibitory effect on glial cells. TLR4 on both DRG neurones and glial cells signalled via a MyD88/NF-κB-dependent pathway to activate inflammatory gene expression (COX-2, IL-1β, TNFα), with no evidence for signalling via the TRIF/IRF3-dependent pathway (1,2). We therefore hypothesise that when nerve injury attenuates this neurone-glial interaction, then DRG glial cells become hyper-responsive to TLR4 agonists and are then responsible for exaggerated inflammatory responses within sensory ganglia.
[This work was supported by a grant from the Research Grants Council of the Hong Kong SAR (GRF476710).]
(1) Tse, K.-H. et al (2014) Neuroscience 279:10-22
(2) Tse, K.-H. et al (2014) Neuroscience 267:241-251

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The Role of TLR4 and its Modulators in Obesity-related Cardiometabolic Syndrome

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Chronic inflammation is a central mediator that links obesity with a cluster of cardio-metabolic diseases, including insulin resistance, diabetes, vascular disorders and non-alcoholic fatty liver disease (NAFLD). Recent studies from both our laboratory and others have demonstrated a critical role of toll-like receptor-4 (TLR4) in the pathogenesis of obesity-related inflammatory diseases. Inactivation of TLR4 by either genetic or pharmacological approaches is sufficient to prevent endothelial dysfunction and atherosclerosis induced by obesity and diabetes, possibly by reducing NADPH oxidase–indued oxidative stress. Furthermore, ApoE(-/-)/TLR4(mut) mice lacking functional TLR4 are resistant to high fat diet-induced liver inflammation and injury and are less susceptible to the diet-induced production of reactive oxygen species (ROS) and proinflammatory cytokines. In ApoE-deficient mice, X-box binding protein-1 (XBP-1), a transcription factor involved in the unfolded protein responses, is activated in the liver by high fat diet, whereas XBP-1 activation is abrogated in ApoE(-/-)/TLR4(mut) mice. In primary rat Kupffer cells, endotoxin induces XBP-1 activation through ROS production, whereas siRNA-mediated knockdown of XBP-1 expression results in a marked attenuation in endotoxin-evoked NF-κB activation and cytokine production, suggesting a key role of TLR4 siganing in mediating the progression of simple steatosis to steatohepatitis, by inducing ROS-dependent activation of XBP-1. Adipocyte fatty acid-binding protein (A-FABP), which is highly expressed in both adipocytes and macrophages, forms a positive feedback loop with TLR4 to potentiate obesity-induced inflammatory responses. The pharmacological inhibitors of A-FABP have been shown to be effective for treatment and prevention of several obesity-related inflammatory diseases, including vascular inflammation, atherosclerosis, insulin resistance and NAFLD. (*Acknowledgement: supported by Hong Kong RGC, C7055-14G*)

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"Toll" Of Knowing You Are Sick: Implications For Pain And Addiction

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The aetiology of persistent pain in humans is comprised of a complex, twisted and multi factorial journey that culminates in a "cancer of the soul". Recent advances in the basic science underpinning our mechanistic understanding of persistent pain have embraced "the other brain" as an integrator of multiple life stimuli. This complex integration of life experiences, which are translated into neurokine signals cause the neuroimmune cells of the central nervous system to adapt and change the environment in which the neuronal system operates. If these adaptations present in the somatosensory neuroanatomical locations then this can present as hypernocicpetion and eventual persistent pain. Our appreciation for this neuroimmune signalling and its contributions to the health and disease of the brain has its origins in the study of the illness response. It is now apparent that these specialised brain-immune processes are engaged in a range of other disparate responses, including the rewarding properties of drugs of abuse. This presentation will summarise recent studies in this field and equip the attendees with further insights of the complexity and power that viewing the brain as a neuroimmune organ brings to understanding persistent pain and drug responses.

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Is Morphine-Induced Emesis TLR4-Dependent?

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Morphine is a valuable drug for the treatment of severe pain. The useful analgesic properties and the side effect of emesis have been ascribed to an activation of opioid receptors (1). Recently, morphine has been recognised to also stimulate Toll-like receptor 4 (TLR4) but the role of these receptors in its gastric side effects has not been explored. In the present investigation, we compared morphine with the TLR4 activator, lipopolysaccharide (LPS), on gastric-, cardiovascular-, and temperature-homeostatic mechanisms, coupled with immunohistochemistry to assess patterns of neuronal cell activation.

Male ferrets were implanted with telemetry devices to record gastric myoelectric activity, blood pressure and temperature. Seven-days later, they were injected subcutaneously with morphine (2 $mg \cdot kg^{-1}$), LPS (2 $mg \cdot kg^{-1}$), or saline (0.5 $ml \cdot kg^{-1}$). After 4 h, animals were killed and tissues removed for processing.

Morphine induced emesis following a latency of ~3 min and was associated with tachygastria, followed by bradygastria. LPS was not emetic and produced a sustained increase of tachygastria; it also induced defeacation. Only LPS increased blood pressure, but both treatments increased heart rate; the effect of LPS was also associated with a reduction of heart rate variability (HRV). Morphine produced a small increase of core body temperature, whereas LPS produced long lasting hyperthermia. Morphine increased c-Fos expression in the thalamus in contrast to LPS, which increased c-Fos expression in the hypothalamus. LPS, but not morphine, decreased the numbers of lymphocytes, neutrophils and platelets in blood and increased serum TNFα levels.

In conclusion, some of the actions of morphine, including an ability to rapidly induce emesis, were different from LPS, and are probably TLR4-independent. Further studies with selective TLR4 antagonists are required to fully elucidate the mechanisms involved.

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Mechanism-based Systemic Approach in Ethnic Sensitivity Assessment to Support China Bridging Studies

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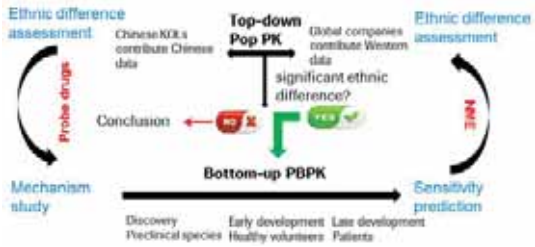

Assessing sensitivity of a medicine's pharmacokinetics /pharmacodynamics (PK/PD) to ethnic factors is key to extrapolate safety and efficacy from one region to a new region. Currently in China and Japan, due to lack of understanding the underlying mechanism of ethnic differences, almost all drugs, at some point of development, need to be assessed for ethnic sensitivity by conducting a PK/PD study in East Asian population, consuming significant amount of resource and time. We propose a combined top-down and bottom-up approach (see figure below) to systematically study the ethnic sensitivity starting with PK. A corporate-academic consortium has been formed aiming to set up a precompetitive and collaborative platform to enable data integration across ethnic groups with a focus on the ADME targets and the physiological parameters. Top down approach utilizes observed Caucasian and Asian PK data in probe drugs with well-defined ADME pathway, and to gain quantitative information on ethnic effect size in each process using population PK analysis. Leveraging the learning from the top-down approach, we will look into the underlying mechanism, such as genetic variability and/or expression differences, for those pathways with large ethnic differences. The mechanistic understanding will serve as the input for the bottom-up approach together with population physiology parameters to predict ethnic sensitivity for novel drug candidates starting at preclinical stage. The prediction will guide the early planning of ethnic bridging studies, be validated when human data sets are available at various stages and support labeling. A number of case studies will be discussed to illustrate the paradigm shift from empirical, fragmental and molecule-based to a systemic, mechanism based PBPK approach for ethnic sensitivity prediction.

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The Important Role of Virtual Chinese Population for PK/PD Prediction: Perspective View from Academic Institution

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Introduction. Virtual clinical trials which make the earlier phase drug development more efficient and safer are knowledge-based study. The fundamental work is to build up virtual population. Virtual population will be wildly applied in following three major areas. First, in first in human studies, it helps to select starting dose and design dose escalation plan. Second, it can play important role in bridging study to predict PK difference of ethnicities and special populations. Third, by integrating patients' information, it can be used to design phase 2 protocols. To date, there is a huge gap between real and virtual system. As an academic institution, PUMCH hopes to put our effort on filling that.
Aims. To obtain liver related systematic parameters, e.g. the activities of cytochrome P450 isozymes by using in vitro and in vivo methods, renal related systematic parameters, e.g. glomerulus filtration rate, and some drug targets related parameters, e.g. proton pump of parietal cell in Chinese population.
Methods. Learn-confirm cycles: new systematic parameters from the studies were integrated to the model first and then comparisons between predicted data and observed data from clinical trials were conducted to verify them.
Results. The metabolisms of tool drugs were studied in vitro and in vivo, which reflect the metabolic capability of CYPs, e.g. 3A4 and 2D6 in Chinese population. In addition, a population pharmacokinetic model was developed to quantitatively assess the effect of renal function on the PK profile of Ceftizoxime mainly eliminated through glomerulus filtration in Chinese population with varying ages.
Discussion. Currently commercially available PBPK models use parameters gathered mainly in a typical Western population, Caucasians. However, the main gap is not the software, but rather in the basic knowledge of the ethnic variation of demographic, physiological, enzymatic factors and other intrinsic and extrinsic factors. The Roche-PUMCH fellowship program is proposed to study system parameters and build up PBPK models in Chinese population to fill the gap step by step.

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Inter-Ethnic Differences Or Similarities Of Oncology Disease Models

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Optimal dose and dosing regimen selection in oncology remains empirical. Early clinical studies (Phase 2) are often not designed and analyzed to maximize learning and decisions are often based on limited information (overall response rate, ORR or progression free survival, PFS) resulting in a high failure rate in Phase 3 trials.
A drug-disease modeling approach has been successfully applied to predict expected clinical responses and survival in cancer patients in a number of clinical settings (1). This modeling framework focuses on efficacy, and the core of this framework is a tumor growth inhibition (TGI) model that makes use of the full longitudinal tumor size. Tumor size response metrics e.g. change from baseline or response categories at an early visit, growth rate, time to tumor to regrowth are used as biomarkers of drug effect to predict overall survival in drug-independent models. This modeling framework (drug-specific TGI model coupled with drug-independent survival models) can enhance learning from early clinical studies compared to the traditional approach of estimating ORR and PFS. Disease model relating tumor growth inhibition to overall survival in Western and Chinese patients in several solid-tumor diseases will be presented and discussed (2, 3).
Drug-disease models and clinical trial simulation offer a powerful science-based quantitative approach to predict expected drug profile, support end-of-phase 2 decisions, and design and analysis of phase 3 studies in oncology (1).
1 – Bruno R, Mercier F, Claret L. Clinical Pharmacology and Therapeutics, 95, 386-393, 2014
2 – Claret L, Gupta M, Han K et al. J Clin Oncol. 31, 2110, 2013.
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Differences in Cytochrome P450-Mediated Pharmacokinetics between Chinese and Caucasian Populations Predicted by Mechanistic Physiologically Based Pharmacokinetic Modelling

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Table 1 – Recent label cases where the approved drug label information was influenced by PBPK Model in the absence of any dedicated clinical data

Company	Drug Name	Indication	No Clinical Studies

The necessity (or otherwise) of bridging studies depends on the ethnic make-up of the population which the approval was obtained, and the disparities with the make-up of the population in the new target territory for extension of the label. ICH guidelines have emphasized the need for better understanding of such influences to rationalise conduct of any clinical studies. Barter et al. (2013) have shown that the significant differences in Chinese vs Caucasian populations (the frequency of polymorphic CYP enzymes and their abundance, liver weight) can be used within the framework of physiologically-based pharmacokinetics (PBPK) to predict the observed differences in plasma drug concentration–time profiles between these ethnic groups. PBPK has been used to make several statements in some recent approved drug labels (Table 1) without conduct of any dedicated studies. Increasing the confidence in virtual population of patients to adequately represent the real population may shape the "Tomorrow's Medicines". Achour et al (2) have indicated the inter-correlation between different biological attributes (such as enzyme and transporter abundances) is a key factor. The position papers by FDA (3) and EMA (4) on best practices in PBPK are manifestation of the acceptability of PBPK in once their reliability is established.

(1) Barter et al (2013) *Clinical Pharmacokinetics* 52:1085-100 ; (2) Achour et al (2014) *Drug Metabolism and Disposition* 42:500-10 ; (3) Wagner et al (2015); CPT:PSP; *in press ; (4)* Shepard et al (2015); CPT:PSP; *in press*

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Duration of dual antiplatelet therapy after drug-eluting stent implantation: Meta-analysis of large randomised controlled trials

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Introduction. Patients receive dual antiplatelet therapy (DAPT) for 6-12 months after implantation of drug-eluting stents (DES) to prevent stent thrombosis (ST) and myocardial infarction (MI). The efficacy and safety of prolonged DAPT after DES implantation has been questioned.

Aims. The aim of this meta-analysis was to compare safety and efficacy of different durations of DAPT.

Methods. We searched for publications, recent cardiology conference abstracts and ClinicalTrials.gov on randomised trials comparing different durations of DAPT after DES implantation. For inclusion, the report had to report the frequency of cardiovascular and bleeding events. Odds ratios and 95% confidence intervals were estimated using RevMan 5.3.4.

Results. Ten trials were included in the meta-analysis. Results are summarised in the table below.

Endpoints	30 months DAPT vs. 12 months DAPT	12 months DAPT vs. 6 months DAPT	12 months DAPT vs. 1-3 months DAPT	Overall
Myocardial Infarction	0.58 (0.40-0.84)	0.88 (0.64-1.19)	0.90 (0.60-1.35)	0.75 (0.58-0.97)
Stent Thrombosis	0.35 (0.20-0.62)	0.74 (0.42-1.32)	1.00 (0.49-2.06)	0.59 (0.36-0.95)
Stroke	0.93 (0.66-1.31)	1.12 (0.56-2.27)	1.00 (0.43-2.32)	1.00 (0.77-1.31)
Cardiac Death	1.12 (0.73-1.71)	0.91 (0.60-1.38)	1.17 (0.72-1.90)	1.05 (0.83-1.32)
All-cause Death	1.30 (1.02-1.66)	1.06 (0.79-1.42)	1.11 (0.75-1.65)	1.18 (0.99-1.40)
Major Bleeding	1.60 (1.22-2.11)	2.12 (1.17-3.84)	1.65 (0.80-3.41)	1.67 (1.34-2.09)

Discussion. There is now evidence to support the efficacy of DAPT beyond 12 months after DES in preventing MI and ST, but the risk of major bleeding needs to be addressed. Prolonged DAPT does not appear to alter the risk of stroke or mortality. Risk and benefit assessment is needed for prolonged DAPT.

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Hypertension Related Variant Of SLC39A8 Gene Influences Cadmium Uptake and Cell Toxicity

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Introduction. *hSLC39A8* (human solute carrier family 39 member 8) encodes a co-transporter of divalent heavy metal cations, such as Cd^{2+} (1), with elusive physiological role. Recent GWAS have identified a non-synonymous single nucleotide polymorphism rs13107325 to be associated with hypertension (2).
Aims. To investigate the functional impact of rs13107325 resulting in an amino acid substitution from Ala to Thr (A391T) in SLC39A8 on Cd^{2+} transport and downstream signalling pathways.
Methods: Intracellular Cd^{2+} uptake was measured in HEK293 cells overexpressing SLC39A8 (Measure-iT™ Pb and Cd assay kit), and human umbilical vascular endothelial cells (HUVECs) of different genotypes. Cd^{2+}- and genotype- dependence of ERK1/2 and NF-κB pathway's activation were investigated by western blotting and dual-luciferase reporter assay. Cytotoxicity was measured by the lactate dehydrogenase assay.
Results. Overexpression of Ala variant in HEK293 resulted in higher Cd^{2+} uptake and higher cytotoxicity as compared with the Thr variant (Fig.1). This is associated with increased phosphorylation of ERK1 and NF-κB activation. Similar trends were observed in HUVECs with endogenous SLC39A8.
Discussion. Increased Cd^{2+} uptake by SLC39A8 Ala variant is associated with higher cell death in human kidney and endothelial cells. Therefore its altered function due to rs13107325 may indicate a potential therapeutic target in hypertension.

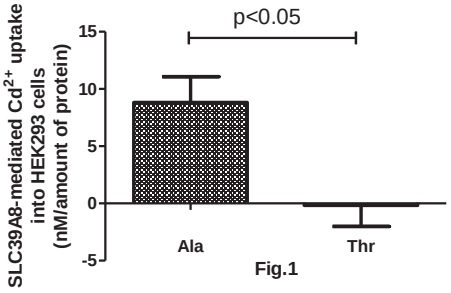

Fig.1

(1) Lei H et al (2006) Mol Pharmacol. 70:171-180
(2) Ehret G B et al (2011) Nature. 478(7367): 103-9.

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Blood Level Of Fibroblast Growth Factor 21 And Blood Pressure

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Introduction: Fibroblast growth factor 21 (FGF21) is a circulating peptide playing a key role in metabolism, obesity and diabetes. An FGF21 analogue has been shown to reduce body weight and ameliorate lipid profile in man. We previously reported that elevated FGF21 blood level was associated with carotid atherosclerosis. We therefore sought to investigate its relationship with blood pressure.
Methods: We measured FGF21 in the plasma of 1921 participants (891 men, 1030 women; 52±12 years) of the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) taken at baseline using an enzyme-linked immunosorbent assay (Antibody & Immunoassay Services, University of Hong Kong). The log of FGF21 level was analysed for relationship with systolic and diastolic blood pressure (BP) at baseline and at follow-up (median 5.4 years).
Results: Plasma FGF21 level was 224.3±7.4 and 214.1±7.1 pg/ml in men and women respectively. It correlated significantly ($p<0.001$) with age ($r=0.30$), waist circumference ($r=0.31$), systolic BP ($r=0.32$), diastolic BP ($r=0.22$), triglyceride ($r=0.41$), HDL-C ($r=-0.27$), fasting blood glucose ($r=0.27$) and hsCRP ($r=0.27$). In multivariate analysis, FGF21 was independently related to systolic ($\beta=0.076$, $p<0.001$) and diastolic ($\beta=0.074$, $p=0.001$) BP at baseline and to diastolic BP at follow-up ($\beta=0.06$, $p=0.025$).
Conclusions: FGF21 blood level increases with age and is related to the components of the metabolic syndrome. It is related to systolic and diastolic BP, independent of age, obesity, lipids and blood glucose. FGF21 may have a role in the pathophysiology of hypertension.

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ARISTOTLE's Message on Mortality in Apixaban Treated Patients with Atrial Fibrillation

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Introduction. ARISTOTLE (1) was a double blind, double dummy, randomised clinical trial in patients with atrial fibrillation and one other risk factor for stroke, that recorded important endpoints during oral therapy with *apixaban* (a non-vitamin K antagonist) 5 mg taken twice daily or *warfarin* (targeted at achieving an international normalized ratio [INR] of 2.0 to 3.0). *Apixaban* as opposed to warfarin therapy was reported to confer significantly greater efficacy in terms of stroke (ischaemic & haemorrhagic)/embolic event prevention, safety, and overall mortality, whilst also avoiding the need to monitor interference with clotting. Whereas the benefits and prevention of harm accruing for important study endpoints including mortality appeared to be impressive when described in relative terms, expressing them in absolute terms is more clinically relevant.
Aims. We therefore set out to also determine possible absolute effects of apixaban compared to warfarin.
Methods. As previously described (2), we derived unadjusted relative risk (RR) and number needed to treat (NNT)/year values for prophylaxis with apixaban versus warfarin for several critical endpoints.
Results. (See table)

Patient Numbers (median follow-up)	Selected endpoint	%RR (95% CI)	NNT/year (95% CI)
Apixaban* 9120; Warfarin 9081 (1.8 years) *dosage reduced in certain patients	Stroke/systemic embolism	80 (66 to 96)	303 (169 to 1501)
	Hemorrhagic stroke	51 (35 to 75)	428 (273 to 987)
	Any death	90 (80 to 100)	238 (119 to ∞)

Discussion. For these endpoints all RRs were favourable, but paradoxically the absolute mortality benefit (NNT/year) exceeded that for stroke/systemic embolism. This suggests that compared to warfarin, apixaban prevented deaths additional to those accruing from stroke/systemic embolism prevention.

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An Online Repository Of Free Educational Resources To Support Practical Pharmacology Teaching

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Introduction. Computer-based simulations of pharmacological preparations are increasingly used to supplement or replace traditional practical classes. For most faculty developing such resources is beyond their skills and available time so the only option is to use third-party resources which may not meet completely their specific teaching objectives.
Aims. to develop a freely accessible, online repository of quality assured learning objects designed to support pharmacology practical teaching. These will support teachers to develop their own resources tailored to their teaching needs.
Results. The Virtual Pharmacology Lab (www.virtualpharmacologylab.com) is an open access repository containing >650 learning objects. These were obtained by disaggregating eleven existing computer-based simulations, (developed by Dewhurst - see www.sheffbp.co.uk), into their component elements. Each learning object is tagged with descriptive metadata including author, title, and brief description and include: data traces – e.g. tissue response to administration of a drug/drug combination; textfiles - e.g. description of how a particular experiment is performed and the apparatus used; images, diagrams; video – e.g. dissection of a preparation, setting it up in an organ bath; interactive student tasks and self-assessment questions.
Discussion. Making learning objects available in more granular formats will enable teachers to incorporate them into e.g. a website, PowerPoint presentation, e-book. It is hoped that colleagues will work with the authors by contributing additional resources and make these freely available for (non-profit) teaching purposes under the same Creative Commons license.

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Pharmacy Education Enhanced By Simulated Immersive Learning Clinical Scenarios (SILCS)

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Introduction. Currently, training for over-the-counter medicines provision is delivered to students in a traditional didactic manner, to achieve competency in the supply of pharmacy-only and pharmacist-only medicines. Healthcare education is progressively integrating simulation technology to promote active engagement and outcome-based learning, and for the ability of simulation to replicate authentic professional practice scenarios.
Aims. This study aimed to investigate the effects of a novel interactive simulation tool for measuring engagement and competency, designed for second-year pharmacy students, to develop over-the-counter medicines knowledge, whilst replicating the pharmacy environment and patient interaction.
Methods. Incorporation of several technologies allowed creation of the interactive simulations, which were delivered on Wix™. The cases were interactive and multidirectional. Engagement and competency was evaluated using click-tracking and eye-tracking software. Surveys were completed pre- and post- tool use, measuring perceived satisfaction and engagement, competency in the provision of over-the counter medicines, and to provide feedback. Student engagement and performance results examined the tool's efficacy as a learning resource. Data were analysed using paired t-tests on Graphpad InStat software.
Results. Pilot studies demonstrated consistently positive student responses and feedback. Students found the tool to be valuable and highly engaging. Statistically significant improvements ($P<0.05$) were demonstrated in counselling knowledge and confidence in questioning, referral and product choice.
Discussion. The simulation tool for the provision of over-the-counter medicines education, encourages student engagement and promotes positive learning outcomes. Furthermore, preliminary results suggest the tool has value as a viable measure of competency assessment in this field. Future research will include facial analysis to establish a stronger engagement measure.

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Health Professionals' Perspectives Of Electronic Prescribing And Medication Management In Residential Care

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Introduction. A novel integrated electronic prescribing and medication management system (E-PMMS) was implemented at a residential aged care facility (RACF) in Victoria, Australia. It enabled general practitioners (GPs) to prescribe medications electronically, both at the facility and remotely, with medication orders auto-populating the RACF's electronic medication administration chart (RACF-EMAC) and communicated electronically to the RACF's pharmacy.
Aims. To explore health professionals' experiences with and perceptions of the E-PMMS.
Methods. Three months after implementation, two focus groups were conducted: one with GPs (n=5) and one with RACF nurses and managers (n=12). An in-depth interview was conducted with one pharmacist, and a second provided responses via a self-administered questionnaire. Data were transcribed verbatim.
Results. Several benefits of E-PMMS were identified including: electronic and remote prescribing enabled GPs to avoid some unplanned visits to the RACF; direct transmission of medication orders to the pharmacy and RACF-EMAC reduced the need for faxed copies and reduced medication discrepancies due to illegible orders or transcription errors. Barriers identified included: slower prescribing and less flexibility with prescribing or editing medication orders compared to paper medication charts; inability of pharmacists to view the RACF-EMAC; incomplete uptake of E-PMMS by GPs, resistance to use of E-PMMS from some GPs, and inadequate training or support.
Discussion. E-PMMS has the potential to improve medication safety, workforce efficiency and interdisciplinary communication in medication management. However, to fully realise its benefits, adequate training and support and greater uptake by GPs will be needed.

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Enhancing Medication Safety For Older People Receiving Medication Support from a Community Nursing Service (CNS)

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Introduction. Many older Australians living at home receive medication support from CNSs. Increasing polypharmacy has led to increased medication management complexity and risk of adverse events.
Aims. To identify medication risk-factors and pilot a pharmacist-led intervention for CNS clients.
Methods. Retrospective case note review for 100 older people referred to a CNS for medication support. Data included clients' health, medication management and use of services including General Practitioner (GP)-initiated Home Medicines Review (HMR). An intervention was developed and piloted, in which CNS nurses referred at-risk clients to a CNS-employed pharmacist for medicines reconciliation, medication management review and consultation with the client's GP, community pharmacist and community nurse to resolve medication-related problems (MRPs) and ensure all had access to an accurate medicines list.
Results. Medication risk-factors included: multiple health conditions (median=5); polypharmacy (median=7 medicines); 'high risk' medicine(s) (48%); cognitive impairment (38%); medication incident(s) (41%); adverse drug reaction(s) (ADRs) (7%); multiple medication lists (treatment authorities) (17%); poor uptake of HMR (5%). Over an 18-week pilot period, 31/38 clients referred to the CNS pharmacist received the intervention (median age 86 years) and 23 consented to evaluation. The pharmacist identified 95 MRPs (median=4 per client; range 2−7), including: errors/discrepancies on treatment authorities (21%), complex medication regimen (possible simplification) (11%), inappropriate storage/hoarding/expired medicines (10%), poor adherence to medicines (8%), potential drug-drug interactions (8%), unnecessary therapy (8%), potential ADRs (6%), incorrect administration technique (5%), potentially inappropriate medicines (4%), duplicated therapy (2%), suboptimal therapy (2%) and others (6%).
Discussion. CNS clients were at risk of medication misadventure. Preliminary findings of the pilot demonstrated that MRPs were highly prevalent.

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Engineering A Novel Analgesic µO-conotoxin With Selectivity For $Na_v1.8$

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Introduction. $Na_v1.8$ is preferentially expressed in small-diameter sensory neurons or nociceptors, which are a type of sensory neuron that responds to noxious or painful stimuli, making it a potential target for the treatment of pain. MfVIA is a µO-conotoxin discovered from the venom of *Conus magnificus* that exhibits selectivity for $Na_v1.8$, making it a potential drug lead. However, MfVIA also inhibits $Na_v1.4$, which is an undesirable off-target, as $Na_v1.4$ is expressed in skeletal muscle.
Aims. The aim of this study was to synthesize a range of MfVIA analogues to improve our understanding of the structure-activity relationships of MfVIA and to use this information to engineer analogues of MfVIA with increased selectivity for $Na_v1.8$.
Methods. Nineteen MfVIA analogues were produced by chemical synthesis and activity was assessed in Human Embryonic Kidney (HEK 293) cells heterologously expressing $hNa_v1.1$-1.8 using a fluorescence-based assay ($FLIPR^{TETRA}$). The anti-nociceptive effect of the most selective MfVIA analogue was assessed in multiple mouse models of pain.
Results. An alanine scan of the top eleven surface-exposed residues revealed E5, E8 and Y19 to be the most important residues for activity at $Na_v1.4$ and $Na_v1.8$. This led us to synthesise MfVIA-E5K/E8K, which improved the selectivity of MfVIA for $Na_v1.8$ over $Na_v1.4$ from 2 fold to 46 fold. Activity at the other Na_v subtypes did not significantly increase: pIC_{50} $Na_v1.1$, > -4.52; $Na_v1.2$, -5.12 ± 0.06; $Na_v1.3$, -5.39 ± 0.42; $Na_v1.4$, -4.68 ± 0.12; $Na_v1.5$, -5.47± 0.13; $Na_v1.6$, -5.60 ± 0.14; $Na_v1.7$, > -4.52; $Na_v1.8$, -6.34 ± 0.23. MfVIA-E5K/E8K was anti-nociceptive in formalin, carrageenan and ciguatoxin mouse models of pain.
Discussion. We were successfully able to engineer MfVIA analogues with improved selectivity for $Na_v1.8$ over $Na_v1.4$, making MfVIA-E5K/E8K the most selective conopeptide for $Na_v1.8$ described to date. This analogue was anti-nociceptive in multiple mouse models of pain, making it a promising drug lead.

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Metabolomic Analysis in Amyotrophic Lateral Sclerosis Reveals Presymptomatic Disruption of Sphingolipid Metabolism

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Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset disease characterized by selective degeneration of upper and lower motor neurons, muscle wasting, and paralysis. ALS is associated with hypermetabolism in patients and SOD1 mutant mice and high lipid diet is becoming standard practice.
Aims. Can metabolomic analysis of ~3000 lipids, in mutant SOD1 mice, allow new therapeutic approaches?
Methods. Ultra performance liquid chromatography coupled to time-of-flight mass spectrometry was used to investigate the lipidome of in plasma, spinal cord, soleus muscle and brain of mutant FVB/N mice overexpressing SOD1(G86R), a model of ALS, at pre-symptomatic stage (70 day old) and at onset of paralysis (100 day old).
Results. Important rearrangements of lipids occurred in skeletal muscle and spinal cord (but not brain) even before overt neuropathology. Triglycerides were massively depleted at end stage disease. *In silico* functional analysis revealed highly significant ($p<10^{-7}$) alterations in the metabolism of some sphingolipids. Glucosylceramides and derived glycosphingolipids were changed in spinal cord and muscle of mutant SOD1 mice (presymptomatic). The expression of UDP-glucose ceramide glucosyltransferase (UGCG), responsible for the synthesis of glucosylceramide, was up-regulated in muscle of mutant SOD1 mice and in patients. These findings strongly suggest that sphingolipid metabolism might be critical to ALS pathology and open up therapeutic perspectives.

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Robust Neuritogenesis-promoting Activity By Bis(heptyl)-cognitin Through The Activation Of Alpha-7 Nicotinic Acetylcholine Receptor/ERK Pathway

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Introduction: Neurodegenerative disorders are caused by the progressive neuronal loss in the brain, and hence compounds that could promote neuritogenesis may have therapeutic values.
Aims: In the current study, the effects and mechanisms of bis(heptyl)-cognitin (B7C), a multifunctional dimer, on neurite outgrowth were investigated in both PC12 cells and primary cortical neurons.
Methods: Immunocytochemical staining was used to evaluate the pro-neuritogenesis effects, western blot and short hairpin RNA assays were applied to explore the underlying mechanisms.
Results: B7C (0.1-0.5 μM) induced robust neurite outgrowth in PC12 cells, as evidenced by the neurites-bearing morphology and the up-regulation of growth-associated protein-43 expression. In addition, B7C markedly promoted neurite outgrowth in primary cortical neurons as shown by the increase in the length of β-III tubulin-positive neurites. Furthermore, B7C rapidly increased the phosphorylation of extracellular signal-regulated kinase (ERK). Specific inhibitors of alpha 7-nicotinic acetylcholine receptor (α7-nAChR) and MEK, but not those of tyrosine kinase receptor A, p38 or JNK, blocked the neurite outgrowth as well as ERK phosphorylation induced by B7C. Most importantly, genetic depletion of α7-nAChR significantly abolished B7C-induced neurite outgrowth in PC12 cells.
Discussion: B7C promoted neurite outgrowth through the activation of α7-nAChR/ERK pathway, which offers a novel insight into the potential application of B7C in the treatment of neurodegenerative disorders.

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Sirt1 Expression In The Central Nervous System Is Associated With Fear And Anxiety Responses In Mice

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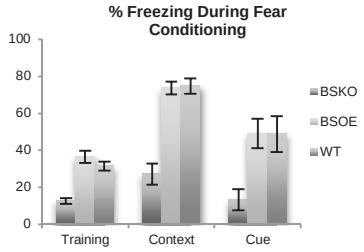
Introduction. Evidence suggests that SIRT1 plays a key role in cognitive and behavioral functions (1).
Aims. This study investigated the effects of a brain-specific SIRT1 knockout and overexpressor mouse model on fear and anxiety responses.
Methods. Brain specific knockout/overexpressor (BSKO/BSOE) mice were generated using the Nestin-Cre system on C57BL6 background. 45-65 week-old BSKO, BSOE and wild type (WT) mice underwent fear conditioning (FC) and elevated plus maze (EPM) testing to determine the impact of SIRT1 expression in the central nervous system on memory formation, fear, and anxiety responses.
Results. The results of FC showed a marked reduction in freezing behavior in the BSKO group, while there was no significant difference between the BSOE group and WT. EPM testing showed that the BSKO group had decreased speed and increased distance-moved compared to WT, while the BSOE group had decreased speed and decreased distance-moved compared to both BSKO and WT groups.
Discussion. Together, these findings show that BSKO of SIRT1 is linked with decreased fear and anxiety responses, while SIRT1 BSOE mice behave similar to WT mice. The impact of SIRT1 on fear conditioning in particular suggests an importance in spatial and contextual memory formation. Understanding the relationship between ageing, SIRT1 expression and behavior can provide new target for therapeutic management of fear and anxiety.



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Pharmacology of the Novel Redox Signalling Molecule Nitroxyl (HNO): Cyclic GMP-Dependent and –Independent Actions

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Introduction. Nitroxyl (HNO), the reduced and protonated congener of nitric oxide ($NO^{\bullet}$), is rapidly emerging as a novel entity with distinct pharmacology and therapeutic advantages over $NO^{\bullet}$ (1). Unlike $NO^{\bullet}$, HNO interacts directly with thiols to increase myocardial contractility, elevates plasma levels of calcitonin gene-related peptide and is resistant to scavenging by superoxide ($^{\cdot}O_2^-$). Whilst these unique properties of HNO confer potential in the treatment of heart failure, our studies have also highlighted vasoprotective actions of HNO.
Aims. To investigate the vasoprotective actions of HNO donors in health and disease.
Methods. Vasoprotective actions of HNO were studied in models of hypertension and atherosclerosis.
Results. Our studies have shown that like $NO^{\bullet}$, HNO donors serve as potent vasorelaxants (including human arteries) and inhibit platelet aggregation, mediating these effects predominantly via sGC activation and an elevation in cGMP. In contrast to $NO^{\bullet}$, HNO donors are resistant to scavenging by $^{\cdot}O_2^-$ and vascular tolerance development and target distinct vascular signaling pathways. Indeed we have shown that the vasoprotective (vasorelaxant, anti-aggregatory) actions of HNO are sustained in the setting of hypertension (SHR rat) and atherosclerosis ($ApoE^{-/-}$ mouse), where endogenous $NO^{\bullet}$ bioavailability is reduced. Furthermore, HNO rapidly suppresses vascular $^{\cdot}O_2^-$ generation from Nox2-NADPH oxidase, via a cGMP-independent action, and limits vascular dysfunction (2).
Discussion. HNO donors offer considerable advantages over traditional $NO^{\bullet}$ donors due to their preserved bioavailability in oxidative stress, lack of tolerance development and favourable vasoprotective properties and may provide innovative pharmacotherapy for the treatment of vascular disease.

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Cardiac Targets Of Nitroxyl: Diastolic Function, Remodelling, And Oxidative Stress

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Nitroxyl (HNO), a redox congener of nitric oxide (NO•), is a novel regulator of cardiovascular function. Like NO•, HNO is a vasodilator and anti-aggregatory agent, with these protective actions predominantly cGMP-mediated. NO• and HNO also share the ability to improve left ventricular (LV) relaxation, although HNO may be more potent in this regard as both cGMP-dependent and-independent actions likely contribute to this. HNO also has a unique ability to enhance LV systolic function, which at the level of the isolated myocardium is predominantly cGMP-independent (i.e. in the absence of confounding haemodynamic influences). Our laboratory has demonstrated that classical HNO donors (Angeli's salt, IPA-NO) prevent acute cardiac hypertrophic responses (up to 48h in cardiomyocytes *in vitro*). cGMP-dependent suppression of cardiomyocyte NADPH oxidase and p38MAPK (key triggers of the hypertrophic response) likely contribute to these antihypertrophic actions. HNO also appears to acutely limit cardiomyocyte hypertrophy independently of both NO• and CGRP, even under conditions of elevated superoxide. In the intact, buffer-perfused heart over the short-term, HNO donors elicit dose-dependent enhancement of LV systolic and diastolic function, concomitant with vasodilatation, with contributions from both cGMP-dependent and -independent mechanisms. HNO thus acutely modulates both LV contractile function and LV relaxation, while concomitantly unloading the heart. Using the mixed HNO-NO• donor 1-nitrosocyclohexylacetate (1-NCA), we have recently provided the first evidence of the beneficial effects of chronic therapy with a putative HNO donor over the longer-term *in vivo*, in any cardiomyopathy context. In a mouse model of chronic diabetes, 4 weeks of 1-NCA therapy limits cardiomyocyte hypertrophy and LV superoxide, and rescues LV diastolic function. Collectively, our studies provide the first evidence that HNO donors may represent a promising new strategy for the treatment of diabetic cardiomyopathy, and implies their therapeutic efficacy in settings of chronic heart failure, either as stand-alone therapy and/or as a supplement to standard care.

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Therapeutic Potential of Nitroxyl (HNO) in Acute Heart Failure: From Basic Science to Clinical Evaluation

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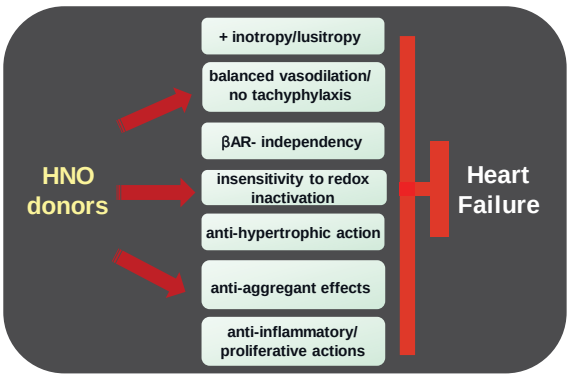

Introduction. Heart failure (HF) remains a major conceptual, therapeutic and social challenge. New therapeutic agents are needed for HF patients in need of inotropic support and unloading effects. **Aims.** Here, I will review the cardiovascular properties of agents releasing nitroxyl (HNO), the one electron-reduction product of $NO^{\cdot}$, and discuss their therapeutic potential in HF patients. **Results.** HNO donors exert positive inotropy/lusitropy in *in vivo* normal and failing animals, while unloading the heart and exerting a balanced (venous *vs.* arterial) vasodilation. These actions occur independently from βAR-activation; they are not subject to tachyphylaxis and are preserved in presence of highly oxidizing conditions. At the cellular level, HNO increases myocyte function via a well-orchestrated enhancement of SR Ca^{2+} release/reuptake and myofilament response to Ca^{2+}, without sizable recruitment of extracellular Ca^{2+} and/or increasing diastolic Ca^{2+}. To achieve these effects, HNO appears to reversibly and likely selectively react with strategically located thiol groups (-SH) in proteins associated with the electro-contraction coupling machinery. **Conclusions.** HNO donors are attractive alternatives or additions to current therapies for patients with acutely decompensated HF. Moreover, HNO's ability to counter *in vitro* (and possibly *in vivo*) cardiac hypertrophy, platelet aggregation, pro-inflammatory and proliferative signaling suggest that this new class of drugs can also benefit patients with chronic HF in which loss of function, vascular accidents and adverse remodeling typically occur.

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Targeting Soluble Guanylate Cyclase For The Treatment Of Cardiopulmonary Disease

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The soluble guanylate cyclase (sGC) is a key signal-transduction enzyme in the cardiovascular system and activated by NO. It became apparent that many cardiovascular diseases are associated with a dysfunction of the NO/sGC system. Importantly, two different forms of sGC exist in vivo, the native and heme-free sGC. sGC activators, such as cinaciguat (BAY 58-2667) are capable of selectively activating the haem-free enzyme via binding to the enzyme's haem pocket. These new compounds selectively target the dysfunctional sGC that is prevalent under disease conditions. Cinaciguat has demonstrated efficacy in patients with acute decompensated heart failure (ADHF), reducing pre- and afterload and increasing cardiac output. sGC stimulators, such as riociguat (BAY 63-2521), show a dual mode of action: they sensitize sGC to the body's own NO while also directly stimulating sGC independently of NO. They may be beneficial in the treatment of a range of cardiovascular and non-cardiovascular disorders. Riociguat had beneficial effects on pulmonary haemodynamics, right heart hypertrophy, and remodeling of the pulmonary vasculature in different experimental models of pulmonary hypertension (PH). In phase III studies riociguat has demonstrated efficacy in patients with pulmonary arterial hypertension (PAH) and, remarkably, also in patients with chronic thromboembolic pulmonary hypertension (CTEPH). Recently, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved Adempas® (riociguat) for use in these two forms of pulmonary hypertension: The treatment of adults with persistent/recurrent CTEPH after surgical treatment or inoperable CTEPH to improve exercise capacity and WHO functional class; and the treatment of adults with PAH to improve exercise capacity, improve WHO functional class and delay clinical worsening.

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Clinical Trials To Understand The Effects Of Prescribing Medicines In Older People

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Introduction. Older people take more medicines, have more co-morbidities and have impaired resilience due to age-associated changes in many organ systems, most marked in frail older individuals. As a consequence older people are more at risk of adverse drug reactions, disease-drug interactions and drug-drug interactions. Thus robust data are needed on the efficacy and risks of medicines in older people. However older people are typically significantly under-represented in randomised controlled trials of medicines, generally considered the gold standard to determine the balance of risks and benefits of drug therapy.

Discussion. Significant progress has been made in removing arbitrary upper age limits from clinical trials but older people are often excluded from trials due to protocol exclusion criteria replating to comorbidities, disability, capacity to consent and the burden participation places on some older participants. Most trials have poor assessment and recording of older participant's baseline status, specifically with respect to frailty. Few data exist on the influence of frailty on drug response and risk of adverse effects. Additional methodologies to assessing risks and benefits of medicines in older people can be used particularly in the period after licensing of new drugs. National and international registries can provide good understanding of serious adverse drug reactions. For example the Safe Implementation of Thrombolysis Database demonstrated the risk of symptomatic intracranial bleeding associated with thrombolytic therapy in over 80 year olds when this group had been excluded from the trials of stroke thrombolysis (1). Similarly case control studies from acute stroke therapy databases demonstrated the odds ratio for a good outcome between thrombolysis treated cases and controls was the same in over 80 year olds as younger patients (2). Understanding of the effects of medicines in older people would be improved with the development of more detailed post marketing study plans and the analysis of databases with more information on characteristics of older people particularly with respect to frailty status.

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Clinical Trials To Understand The Effects Of Deprescribing In Older People

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Introduction. Deprescribing involves the reduction in the number and/or dose of medications under the supervision of a clinician. This is usually in the setting of chronic polypharmacy and multimorbidity, or when there are changes in patient goals such as occur with dementia, frailty, terminal illness or the need for institutional care. Deprescribing is a common practice in geriatric medicine and is performed to improve quality and quantity of life and to reduce adverse drug reactions. However, deprescribing requires an evidence base, ideally randomized deprescribing clinical trials.
Aims. To review current research on deprescribing and appropriate methodologies.
Results. There are many observational studies showing a strong association between medications, polypharmacy and adverse outcomes in older people, which provides the rationale for deprescribing trials. Most deprescribing trials have focussed on discontinuation of single medications such as statins, antipsychotics, diuretics and antihypertensives. A number of clinical trials are underway internationally to confirm one open label unblinded trial (1) showing that reduction of medications in older people with polypharmacy is effective. However such trials are complicated and the subjects are heterogeneous. There are many studies that have shown that it is possible to reduce the number of medications or inappropriate prescribing, however, few have evaluated clinical outcomes. Therefore novel approaches such as N-of-1 trials and quasi-interventional observational trials may be useful, while additional information on stopping medication should be obtained from subjects that stop medications in any clinical trial (2). Several consensus guidelines have been developed to assist clinicians in identifying inappropriate and/or unnecessary medications, and to undertake deprescribing when indicated.
Discussion. A range of clinical evidence is required to support the practice of deprescribing including deprescribing clinical trials, N-of-1 trials and observational studies.

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Pharmacoepidemiological Studies to Understand Patterns of Medicines Use in Older People

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Multimorbidity and polypharmacy are highly prevalent among older people. Over- and under-treatment is common, particularly among those with dementia and in aged care facilities. Medicine selection is complicated by the limited evidence base specific to frail older people. This presentation will explore factors contributing to increasing polypharmacy, the clinical appropriateness of polypharmacy, and the possible need for intervention and ongoing monitoring. The increasing prevalence of polypharmacy may be partly attributable to strict adherence to disease-specific clinical practice guidelines for conditions such as diabetes, hypertension, stroke and myocardial infarction. Long-term preventative medications may have an unfavourable risk-to-benefit ratio in older people. In aged care facilities, polypharmacy has been associated with comorbidity, recent hospital discharge and number of prescribers; and inversely associated with age, cognitive impairment, disability in activities of daily living (ADLs) and length of stay (1). Pharmacological management of pain and depressive symptoms may be clinically appropriate but the potential benefits should be balanced against the risk of adverse drug events, including daytime sleepiness, falls and fractures (2). Minimising the use of unnecessary and potentially harmful medications may maintain health-related quality of life and reduce the risk of hospitalisation.

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Pharmacoepidemiologic Studies to Understand the Outcomes and Safety of Medicines in Older People

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Introduction. At present, there is limited clinical trial evidence to guide prescribing decisions in real-world older patients with drug-drug, disease-drug interactions and geriatric syndromes.
Aims. To evaluate the current evidence from observational studies on outcomes and safety of medicine use in older people.
Methods. Published evidence in relation to the clinical outcomes of medicine use in older people was reviewed.
Results. There is strong evidence from well-designed pharmacoepidemiological studies that multiple drug use and high risk drugs contribute to poor clinical outcomes including development of frailty and dementia in older people. Recent studies have shown that exposure to high-risk drugs including benzodiazepines and cumulative use of anticholinergic drugs increases the risk of Alzheimer's disease (AD). However, research on how drugs influence clinical outcomes in older people who have already developed a geriatric syndrome is limited. In our national-based study of older people with and without AD living in Finland, there was a dose-response relationship between cumulative anticholinergic and sedative drug use and hospitalisation and mortality over 1-year.
Discussion. Until there are robust clinical trials to provide recommendations for older people with multi-morbidity, geriatric syndromes and polypharmacy specifically, pharmacoepidemiologic studies are essential to understand the safety of medicine use in this patient subgroup.

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Patient Based Treatment Guidelines for Older People With Multimorbidity

Prof Darrell R. Abernethy[1,2], Departments of Medicine and Pharmacology and Molecular Science, Johns Hopkins University School of Medicine[1], Baltimore, Maryland, USA; Office of Clinical Pharmacology, Food and Drug Administration[2], Silver Spring, Maryland, USA.

Prevalent medical conditions in older individuals that may benefit from drug treatment include hypertension, diabetes mellitus, osteoarthritis, congestive heart failure, cancer, chronic obstructive pulmonary disease, dementia, depression, and gastro-esophageal reflux disease. Concurrence of two or more of these diagnoses (multimorbidity) is the rule, not the exception (1). Medical treatment for such older patients using disease-specific treatment guidelines frequently results in polypharmacy (5 or more concurrent medications) with medications that are either not appropriate for some of the illnesses or not appropriate when concurrently prescribed (2).
Explicit incorporation of patient specific factors allows identification of potentially conflicting guideline recommended drug therapies. To optimize the drug regimen for the individual patient, prioritization of treatments is necessary. Factors that should be used to inform the prioritization include the following:

1. Effect size of the treatment for the individual that incorporates estimated life expectancy.
2. Impact of the treatment on both quality and quantity of life.
3. Patient preference.
4. Limit of total medication burden to < 5 concurrent medications.

This requires development and testing of the utility of algorithms that explicitly, rather than implicitly, incorporate these factors into individualized patient specific treatment guidelines to improve the rational use of drugs in older patients. This is a hypothesis to be tested whose time has come.

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Patient Based Treatment Guidelines for Older People with Geriatric Syndromes

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Introduction. Older people frequently present non-specifically with geriatric syndromes such as frailty, falls, confusion and incontinence.
Aims. To review the pharmacological management of older people with geriatric syndromes
Methods. A narrative review was performed on (1) the role of medicines in the aetiology, prevention and management of geriatric syndromes; and (2) the impact of geriatric syndromes on the pharmacological management of disease.
Results. While geriatric syndromes are usually multifactorial, medicines, alone or in combination, frequently contribute to their aetiology and pathogenesis. Pharmacological prevention and management of geriatric syndromes is an area of active research. Withdrawal of medicines that contribute to geriatric syndromes can frequently reverse the syndromes. Geriatric syndromes should be considered when prescribing for older people as they may influence treatment goals, pharmacokinetics, pharmacodynamics, safety and efficacy of medicines.
Discussion. Medicines are one of the most reversible causes of geriatric syndromes. Geriatric syndromes may influence clinical pharmacology and therapeutics in older people.

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Education And Training To Improve Prescribing For Older People With Multimorbidity And Geriatric Syndromes

Professor Simon Maxwell. Clinical Pharmacology Unit, University of Edinburgh, UK.

Polypharmacy is defined in various ways but is extremely common in the elderly population: in the UK, around 50% of the population over 70 years of age group are on 5 or more medicines. Polypharmacy is an inevitable, and often appropriate, accompaniment of the co-morbidities associated with older age (e.g. ischaemic heart disease, cerebrovascular disease, atrial fibrillation, diabetes, osteoporosis, urinary incontinence) ideally reflecting current clinical guidelines based on best evidence. However, there is plenty of evidence of so-called 'problematic polypharmacy' leading to unnecessary risk of significant adverse drug reactions and interactions. Increasing numbers of medicines also risk prescribing errors and non-adherence. Furthermore, many patients simply wish to reduce their 'pill burden' whether it is based on sound evidence or not. These wishes can only be tackled by a thorough appraisal of current prescriptions in the light of their benefits and risks in individual patients combined with careful communication around the anticipated goals and outcomes of treatment. Tackling these challenges will require a professional work force that is equipped with the knowledge and skills to address them. This talk will briefly highlight some of the approaches that might promote better training amongst relevant professional groups.

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Impacts Of Oxidative Stress And Nitrosative Stress On Baroreflex Dysregulation: Clinical And Therapeutic Implications

Samuel H.H. Chan. Center for Translational Research in Biomedical Sciences, Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

The central theme of this lecture is that therapeutic intervention is only achievable against pathophysiological, but not pathological conditions. Using as the example is baroreflex dysregulation, which by itself is not a disease per se, although it impacts daily existence of the general populace and in its most severe from is causally related to fatality. Under physiological conditions, the baroreflex provides a rapid negative feedback mechanism that normalizes fluctuations in blood pressure and heart rate induced by environmental insults. Under pathophysiological conditions such as neurogenic hypertension, the baroreflex is rendered dysfunction because of oxidative stress in its brain stem neural substrates. More importantly, this process is reversible and antioxidant treatment is attainable. When baroreflex is defunct under pathological conditions, nitrosative stress in key nuclei of the baroreflex circuit becomes the primary culprit, which leads to brain dead and other forms of fatality. Intriguingly, this process is irreversible, with diminished therapeutic feasibility. Previous and ongoing work from our group has unveiled an intricate interplay of a multitude of signaling molecules at the level of transcription, translation and post-translational modification in its brain stem neural substrates dictates the phenotypical expression of normal, dysfunction or defunct baroreflex; and reversible or irreversible disruption of the functional connectivity between key nuclei of the baroreflex circuit determines its pathophysiological or pathological status. Representative experimental examples from our clinical and laboratory work will be used to illustrate these notions, including some obtained from recent studies using magnetic resonance imaging/diffusion tensor imaging as an investigative tool in mouse disease models. We conclude that the transition from oxidative stress to nitrosative stress bears crucial clinical implications on baroreflex dysregulation and interruption of this transition should be the central therapeutic target.

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Cardiovascular activity of the endogenous GPR55 agonist, L-alpha-lysophosphatidylinositol

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Introduction. The role of the orphan G-protein-coupled receptor GPR55 in cardiovascular regulation is controversial. Recently, we have found that GPR55 serves as a novel vascular target for the endogenous cannabinoid, *N*-arachidonyl ethanolamine, which relaxes mouse mesenteric arteries via GPR55 and Ca^{2+}-activated K^+ channels (1). However, the cardiovascular activity of another endogenous GPR55 agonist, L-alpha-lysophosphatidylinositol (LPI) (2) remains unclear.
Aims. To determine effects of LPI on the vascular tone and blood pressure in rodents
Methods. Wistar rats, wild type C57BL/6J and $GPR55^{-/-}$ mice (male, 3 months) were used for isometric tension recording in isolated arteries precontracted with 10µmol/L methoxamine (α1-adenoceptor agonist) and blood pressure measurement under general anaesthesia (rats: pentobarbital 60mg/kg, i.p. and implanted with intra-arterial catheter; mice: tail cuff method while maintained at 1% isoflurane).
Results. LPI relaxed rat small mesenteric arteries (pEC_{50}=5.8±0.2, relaxation at 30µmol/L: 92±2%) and aorta (at 30µmol/L=45±7%; n=5 for both). Relaxation to LPI in mesenteric arteries from wild-type mice (10µmol/L: 25±6%; 30µmol/L: 81±7%) was greatly reduced in $GPR55^{-/-}$ mice (30µmol/L: 28±11%, n=5-7; $P<0.01$). Precontraction with 60mmol/L KCl, which inhibits K^+ channel function, abolished LPI relaxations in rats and mice. LPI (1mg/kg, i.v.) reduced rat mean arterial blood pressure by 54mmHg (n=5; $P<0.01$), but there was no significant difference between the blood pressure of wild-type and $GPR55^{-/-}$ mice.
Discussion. This study shows that exogenously added LPI has vasorelaxant and hypotensive properties in rodents. These effects are, at least partly, mediated by GPR55 and activation of K^+ channels.

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Deletion Of Repressor Activator Protein 1 Modulates Vascular Function In Mouse Aorta

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Introduction. Repressor activator protein 1 (Rap1) is a telomeric protein which resides within the shelterin complex and docks at chromosomal ends. Besides maintaining chromosome integrity, it also participates in metabolic regulation and body-weight homeostasis. Its role, if any, in vascular responsiveness is unknown.
Aims. The present study investigated whether or not Rap1 deletion affects vascular responsiveness.
Methods. Female Rap1 knockout and wild-type littermates on a C57BL/6N background were used in the experiments (Aged 13-15 weeks, n=5-6). All mice were kept on standard chow. The thoracic aortae from the two groups of mice were dissected and rings with or without endothelium were suspended in wire myographs to determine contractions and relaxations to increasing concentrations of phenylephrine (10^{-9}-10^{-4} mol/L) and acetylcholine (10^{-10}-10^{-4} mol/L; during contractions to 10^{-6} mol/L phenylephrine), respectively. Contractions were expressed as percentage to the reference response obtained with 60mmol/L potassium solution at the beginning of the experiment. Relaxations were expressed as percentage of the pre-contraction to phenylephrine.
Results. Relaxations to acetylcholine were diminished significantly in Rap1 knockout compared to wild type aortae with endothelium (pEC50: 8.13±0.14 vs 7.61±0.08, P<0.001). The E_{max} of contractions induced by phenylephrine was increased in Rap1 knockout aortae with endothelium (E_{max}: 72.51%±2.60 vs 79.54%±1.69, P<0.05). In the absence of endothelium, the contractions to phenylephrine were reduced significantly in Rap1 knockout aortae (pEC50: 6.97±0.13 vs 6.38±0.15, P<0.005).
Discussion. These results demonstrate that Rap1 modulates vascular responsiveness in the mouse aorta. Deletion of Rap1 appears to result in impaired basal and acetylcholine-stimulated release of nitric oxide.

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Differential Effects Of GPR55 On Cardiac Adrenoceptor Subtypes In Mice

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Introduction. Increased cardiac sympathetic activity in heart failure (HF) leads to chronic stimulation and subsequent loss of cardiac β_1-adrenoceptors (β_1-ARs) and reduced AR mediated inotropy [1]. We have previously shown reduced contractile reserve following dobutamine administration in GPR55$^{-/-}$ mice [2]
Aims. To identify the AR subtype(s) affected by GPR55 gene deletion and determine their role in the cardiac decompensation.
Methods. Mice (WT and GPR55$^{-/-}$; 3months old) were anaesthetised with ketamine/xylazine (120mg/kg & 16mg/kg i.p.,) and a 1.4-Fr pressure conductance catheter inserted into the left ventricle to measure pressure volume loops (PVL). Responses to dobutamine (10µg/kg; $\alpha_1/\beta_1/\beta_2$-AR agonist; n=9), procaterol (0.02-2µg/kg; β_2-AR agonist; n=7-8), A-61603 (0.2-20µg/kg; α_1-AR agonist; n=9) and dobutamine (1-10µg/kg) plus prazosin (α_1-AR antagonist) and ICI 118,551 (β_2-AR antagonist, both 1mg/kg i.p; n=9) were assessed in both strains.
Results. GPR55$^{-/-}$ mice exhibited reduced contractile responses to dobutamine alone. GPR55$^{-/-}$ mice exhibted significantly attenuated β_1-AR mediated (dobutamine in the presence of prazosin/ICI 118,551) pressor (ESP; 4±1 vs. 14±1mmHg), lusitropic (dP/dt_{min}; -82±174 vs. -1013±175mmHg/µL), and inotropic (dP/d$t_{max;}$ 1354±128 vs. 2047±145mmHg/µL) & ejection fraction (2±1.2 vs. 12±4%)) responses compared to WT mice (all *P*<0.05). In GPR55-/- mice, A-61603, induced significantly greater pressor (ESP; 77±6 vs. 60±2mmHg), lusitropic (dP/dt_{min}; -3013±312 vs. 205±100mmHg/µL), and inotropic (dP/dt_{max}; 5759±469 vs. 3450±256mmHg/µL) responses compared to WT mice (all *P*<0.05). Procaterol had minimal effects on cardiac function in either strain..
Discussion. Our findings demonstrate that GPR55 influences adrenoceptor function in the heart and may play a role in the altered adrenoceptor signalling characteristic of heart failure.

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Association Of Fibroblast Growth Factor 21 With Microvascular Disease In The Fenofibrate Intervention And Event Lowering In Diabetes Study

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Introduction. Baseline fibroblast growth factor 21 (FGF21) levels can predict total cardiovascular disease events in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study.
Aim. This study investigated the relationship of plasma FGF21 levels with baseline and new on-study microvascular disease (MVD) in patients with type 2 diabetes from the FIELD study.
Methods. Plasma FGF21 levels were measured in 9697 study participants at baseline. Total MVD was defined as the presence of any nephropathy, retinopathy, neuropathy, and/or microvascular amputation.
Results. Higher baseline FGF21 levels were found in patients with baseline total MVD ($P<0.001$). The associations remained significant after further adjusting for confounding factors (OR=1.13 per SD increase in ln-transformed FGF21 levels, $P<0.001$). Among 6465 patients without baseline MVD, 1517 patients developed on-study total MVD over 5 years of follow-up. Higher baseline FGF21 levels were associated with a higher risk of new on-study total MVD after adjusting for confounding factors (OR=1.09 per SD increase in ln-transformed FGF21 levels, $P=0.01$). The addition of FGF21 levels in a model of new on-study total MVD with established risk factors significantly increased the integrated discrimination improvement and the net reclassification improvement (both $P<0.01$).
Discussion. Higher baseline plasma FGF21 levels are seen in patients with type 2 diabetes and established MVD, and predict the future development of new MVD over 5 years of follow-up, suggesting a potential role of FGF21 as a potential biomarker for MVD in high risk patients.

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Genotype Frequencies of Selected Drug Metabolizing Enzymes and ABC Drug Transporters among Healthy Pakistanis

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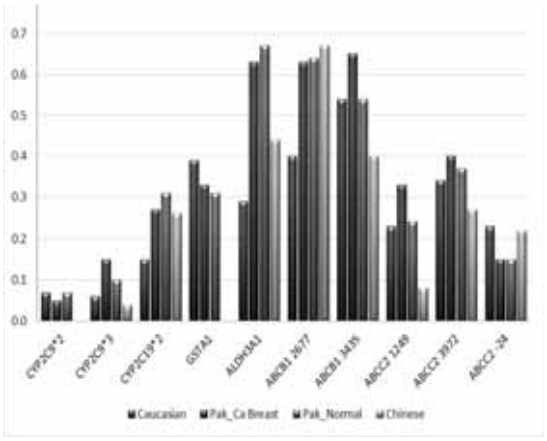
Introduction. The drug response may be influenced by polymorphic genes of drug metabolizing enzymes and transporters (DMET). We previously reported (1) selected genotype profiles in Pakistani patients of breast cancer and their comparison with HapMap.
Aims. To compare previously reported genotype profile among breast cancer patient with healthy Pakistani adults.
Method. Total 155 healthy volunteers (males 36%; females 64%) were genotyped for relevant DMET genes through RFLP or Pyrosequencing using salivary DNA.
Results. The variant allele frequencies (Fig) were: 7% CYP2C9*2, 10% CYP2C9*3, 31% CYP2C19*2, 31% GSTA1*B, 67% ALDH3A1*2, 64% & 54% ABCB1 (2677G>T/A and 3435C>T), and 15%, 24% & 37% ABCC2 (–24C>T, 1249G>A, 3972C>T).
Discussion. In comparison to HapMap database, this study shows in healthy Pakistani population a higher frequency of (a) CYP2C19*2 ($p<0.05$) than Caucasian, (b) ALDH3A1*2 ($p<0.01$) than Caucasian and Chinese, (c) ABCB1 2677G>T/A ($p<0.01$) than Caucasian, and (d) ABCC2 1249G>A ($p<0.01$) than Chinese. These results are not significantly different from our previously reported cancer patients.



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Alternative Methods for CYP2D6 Phenotyping: Comparison of Dextromethorphan Metabolic Ratios from AUC, Single Point Plasma and Urine

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Introduction. CYP2D6 is a high polymorphic enzyme. Determining its phenotype before CYP2D6 substrate treatment can avoid dose-dependent adverse events or therapeutic failures.
Aims. Alternative phenotyping methods of CYP2D6 are compared to evaluate the appropriate phenotyping method after single- and multiple-dose of dextromethorphan and to explore the cut-off values for potential sampling methods.
Methods. 21 subjects were assigned to receive a single dose of dextromethorphan 30mg orally, followed by a 3-day washout period prior to administration of dextromethorphan 30mg Q12h for 6 days orally.
Results. In the single-dose study, statistically significant correlations were found between $MR_{DEM/DOR}$ from AUC_{∞} and from serial plasma points from 1 to 30 h or urine (each $P < 0.001$). In the multiple-dose study, statistically significant correlations were found between $MR_{DEM/DOR}$ from $AUC_{0\text{-}12h}$ on Day 6 and $MR_{DEM/DOR}$ from serial plasma points from 0 to 36 h after the lasting dosing or urine (each $P < 0.001$). Based on reported urinary antimode and linear regression analysis, the antimodes of AUC and plasma points were derived to profile the trend of antimodes as the drug concentrations changed.
Discussion. $MR_{DEM/DOR}$ from plasma samples had good correlations with $MR_{DEM/DOR}$ from AUC, suggesting one plasma point could be used to determine phenotype of CYP2D6. Appropriate sampling time window and corresponding cut-off values were derived for plasma phenotyping method.

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Pharmacokinetic Interactions Between Cyclosporine and Mycophenolate Mofetil in Renal Transplant Patients

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Introduction CsA inhibits the enterohepatic (re)circulation of mycophenolic acid (MPA) and its inactive mycophenolic acid glucuronide (MPAG) metabolite resulting in significantly lower dose-corrected MPA concentrations in CsA-treated patients and early clinical MPA under exposure in 50% [1]. *Objective* To assess drug-drug pharmacokinetic interaction and co-administered drugs influence for dosing.
Methods 12 hour total serum concentration-time profiles of MPA and 12 hour total blood concentration-time profiles of CsA were obtained after an oral administration. MPA concentration was determined by a validated HPLC method and CsA - by using a LC-MS method. The $AUC_{(0\text{-}12)}$ was calculated using a Bayesian estimator and a 3-point limited sampling strategy.
Results The study included 3 groups of patients (postransplantation time >1 year): receiving CsA and MMF (n=21 patient; CsA 200 mg/day and MMF 2000 mg/day) (i), receiving only CsA (n=9 patients; 200 mg/day) (ii) and receiving only MMF (n=7 patients; 1000 - 3000 mg/day) (iii). C_0, $AUC_{(0\text{-}12)}$, C_{max} were compared between the groups. CsA pharmacokinetic parameters were compared between the first and the second group; the MMF pharmacokinetic parameters were compared between the first and the third group. There were no significant differences. Only MMF dose was 28.6% higher in MMF monotherapy group.
Conclusions The results show that CsA affects MMF dosing and increases it 28.6%. No significant differences between pharmacokinetic parameters were noticed. It might be thought that enterohepatic recirculation of MPAG might have less influence on MMF metabolism than MMF is prescribed with CsA.
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High Quality Warfarin Treatment By An Anticoagulant Centre In Queensland, Australia

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Introduction. Warfarin decreases stroke risk in patients with non-valvular atrial fibrillation (NVAF). The benefit of warfarin treatment is largely dependent on the quality of control as measured by the time in therapeutic range (TITR) (1). A minimum target threshold of 60-65% is recommended but this can vary considerably according to clinical setting and region (2).
Aims. The aim of this study was to determine the quality of warfarin therapy by an anticoagulant centre in Queensland using TITR as a measure of control.
Methods. Retrospective data was collected from a major private pathology clinic in Queensland for patients receiving warfarin management between 2009 and 2014. Patients receiving warfarin management for NVAF were identified and the TITR calculated using the Rosendaal method.
Results. Of the almost 8000 warfarin patients managed by the clinic, 3954 of these were being treated for NVAF. This group had a mean TITR of 80.4% and a mean number of tests in therapeutic range of 74.0%. The average time of testing was 1511.6 days with an average testing interval of 15.24 days.
Discussion. The observed mean TITR of over 80% is far superior to the minimum target threshold of 65%. One other Australian study reported a mean TITR of 69.1% (3), also above the target level. A similarly high TITR above 75% was reported by a Swedish anticoagulation centre (4) suggesting the best warfarin control is achieved by dedicated clinics. The high TITR achieved by the Queensland anticoagulant centre demonstrates that dedicated anticoagulant programs can produce high quality warfarin management.

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Topically Applied Tocotrienol (TT) Delays Cataractogenesis By Reducing Lens Oxidative Stress In Rats With Streptozotocin (STZ)-Induced Diabetes

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Introduction. Cataract, the second leading complication of diabetes mellitus in Malaysia, is currently treated only by surgery. Surgery carries cost and complications, hence search for medical treatment is valuable. Since oxidative stress (OS) plays a major role in cataractogenesis, we studied the effect of TT, an analogue of vitamin E, which is known for its antioxidants properties.
Aims. To evaluate the effect of topically applied TT on cataractogenesis and lenticular OS in diabetic rat.
Methods. 150-200g Sprague Dawley rats were divided into 3 groups (n=18). Group 1 was administered with citrate buffer intraperitoneally (IP) whereas group 2 and group 3 were treated with STZ (65mg/kg body weight, IP) to induce diabetes. Group 3 received topical TT (0.03%) daily for 8 weeks while groups 1 and 2 received vehicle. Lens opacity was monitored by retroillumination technique using portable slit lamp. Subsequently, rats were euthanized and lenses were analyzed for superoxide dismutase (SOD) and catalase (CAT) activities and reduced glutathione (GSH) concentration.
Results. Opacity index (OI) in groups 2 and 3 was significantly higher compared to group 1 throughout the experimental period. However, OI in group 2 was significantly lower compared to group 3 ($p<0.05$). Lens SOD and CAT activities in group 3 were normalized with increment of 1.4- and reduction of 2.1-folds respectively ($p<0.001$) compared to group 2. Lens GSH concentration in groups 2 and 3 were significantly lower compared to group 1 ($p<0.001$), however, it was significantly higher in group 3 (1.25-folds, $p<0.01$) compared to group 2.
Conclusion. Topically applied TT reduces the lenticular oxidative stress and therefore, delays cataract progression in STZ-induced diabetic rat.

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A new approach to treat ischaemic heart disease: adenosine receptor biased agonism

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Introduction. Adenosine A_1 receptor (A_1AR) activation represents a powerful cardioprotective mechanism. Unfortunately, the translation of A_1AR agonists into the clinic has been severely hindered due to on-target adverse effects, including bradycardia, atrioventricular block and hypotension (1). Biased agonism has the potential to overcome the current limitations associated with prototypical A_1AR therapeutics, enabling the separation of desired from adverse effects.
Aims. To quantify the bias of prototypical and atypical A_1AR agonists *in vitro* and investigate the ability of biased agonists to mediate cardioprotection in the absence of adverse hemodynamic effects *in vivo*.
Methods. Fluorescence approaches were used to determine phosphorylation of ERK1/2 and AKT, calcium mobilization, cAMP accumulation and cell survival stimulated by A_1AR agonists in CHO cells stably expressing the human A_1AR. An operational approach was used to quantify ligand bias (2). A Langendorff-perfused isolated rat heart model subjected to ischaemia (30 min) and reperfusion (60 min) was used to determine the influence of A_1AR agonists on heart rate and infarct size.
Results. Prototypical A_1AR agonists did not display significant bias for any of the signaling pathways assessed. In contrast, the atypical agonist, VCP746 (3), was significantly biased away from calcium mobilization. In an isolated rat heart model, both the prototypical agonist CPA and the atypical agonist VCP746 mediated a significant decrease in infarct size. However in contrast to CPA, VCP746 had no significant effect on heart rate.
Discussion. Collectively, these studies demonstrate that “fingerprinting” of biased agonism within a model system has the potential to predict novel and physiologically relevant *in vivo* pharmacology.

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Electrophysiological study of primary epididymal epithelial cells revealed functional activities of epithelial Ca^{2+} channel TRPV6 and Ca^{2+}-activated chloride channel in the rat epididymis

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Spermatozoa undergo the final yet essential maturation development in the epididymis to acquire motility and become functionally competent for oocyte fertilization. Calcium is important for sperm physiology but very little is known about the regulation of calcium homeostasis in the epididymis, although it is known that the fluid therein has a low Ca^{2+} content. A recent report on mice with genetic deletion of TRPV6 showed severe impairment in male fertility with abnormal accumulation of Ca^{2+} in the epididymis, highlighting the key role of TRPV6 for the calcium absorption in this organ. In this study, whole-cell patch-clamp study on primary principal cells of rat cauda epididymidis revealed a Ca^{2+} current with the characteristics matching that of TRPV6, *viz* constitutive activity, time dependent inactivation at hyperpolarizing steps, inwardly rectification, pH-sensitivity, and blockade by lanthanides. Under quasi-physiological conditions, removal of extracellular calcium attenuated the current components consisting of a small inactivation current at hyperpolarizing steps, and an activating current at depolarizing steps – a typical characteristic for calcium-activated chloride channel (CaCC). The La^{3+}-sensitive currents also consisted the component of decaying current at hyperpolarizing potentials, which was instantly inhibited, and the activating current at depolarizing potentials, which was inhibited in a slower manner. The putative CaCC blocker niflumic acid partially inhibited the activating currents, whereas La^{3+} almost abolished the whole-cell currents. *In vivo* luminal perfusion of the cauda epididymal tubule showed a Ca^{2+} re-absorption rate of 2.6 ± 0.1 nmol/cm^2/min, which was dose-dependently suppressed by ruthenium red, a putative blocker for epithelial Ca^{2+} channel and CaCC. This study suggests that a coupling mechanism between TRPV6 and CaCC in the principal cells plays a role in the Ca^{2+} homeostatic regulation in the epididymis.

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SIRT1 Enhances Lipid Storage and Utilization in Adipose Tissues Partly Through Adiponectin-mediated Pathways

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Introduction. SIRT1 is a key energy sensor controlling cellular responses to nutrient availability. Adiponectin is an adipocyte-derived hormone eliciting a wide range of beneficial functions in preventing obesity-related medical complications.
Aims. The present study was designed to investigate the role of adiponectin in SIRT1-mediated regulation of lipid metabolism.
Methods. Transgenic mice with selective overexpression of human SIRT1 in adipose tissues were cross-bred with adiponectin deficient mice to produce four different groups of animals including the wild type mice (WT), mice with the SIRT1 transgene (WAS), mice without adiponectin gene (AKO) and WAS without adiponectin expression (WASAKO). Insulin/glucose tolerance tests, calorimetric respiratory analyses and NMR fat percentage measurements were performed for mice at the age of 8-, 17-, and 30-weeks.
Results. Compared to WT, WAS showed similar body mass (BM) and fat mass composition (FM/BM), but increased net weights and triglyceride contents of adipose tissues. The lipid accumulation in liver was significantly reduced in WAS. Adiponectin deficiency abolished SIRT1-enhanced lipid storage in adipose tissue but had no effects on the hepatic lipid contents in WASAKO mice. Various lipid markers were analysed by a gas-phase mass spectrometer (GC-MS). The results revealed that fatty acid composition and distribution were significantly altered by SIRT1 overexpression and/or adiponectin knockout in adipose tissues, which contribute to afore mentioned metabolic differences.
Discussion. Adiponectin deficiency abolishes SIRT1-mediated beneficial effects on lipid metabolism in mice.

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B Cell Subtypes in Atherosclerosis: Mechanisms and Therapeutic Potential

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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 arteries are the result of complex interactions between LDL-cholesterol, endothelial and smooth muscle cells and cells of the immune system. Multiple immune cells accumulate in lesions including macrophages, NKT cells, CD4+ and CD8+ T cells and B cells. Early studies indicated that B cells are protective by producing low affinity IgM antibodies against oxidised LDL (low density lipoprotein). However, recent advances in B cell biology indicate multiple subtypes of B cells suggesting a more complex role in atherosclerosis. Using an anti-CD20 B cell depleting antibody, we reinvestigated the role of B cells in atherosclerosis using fat fed ApoE-/- mice. 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 cells using BAFF receptor knockout mice and anti-BAFF receptor antibodies we demonstrated their important role in atherosclerotic lesion inflammation and development/progression of atherosclerosis, in part by secreting TNF-alpha and producing pathogenic antibodies. 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 B1a B cells aggravates atherosclerosis. These effects were accompanied by marked reductions in anti-oxidised LDL IgM antibodies, increased lesion apoptotic cell numbers and necrotic core development. TLR4-MyD88 signaling is required for B1a B cell mediated suppression of atherosclerosis. Furthermore, targeting TIM-1 expressed by B1a B cells by administering anti-TIM-1 (RMT1-10) mAb to hyperlipidemic ApoE-/- mice expands the B1a B cell population including TIM-1+IgM+ and TIM-1+IgM+IL-10+ B1a B cell subsets and attenuates atherosclerosis.

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Aortic Adaptive Immune Response In Atherosclerosis

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The adaptive immune system plays a crucial role in atherosclerosis. Several immune cells reside in the vessel wall of normal arteries with accelerated recruitment observed in the pathology. However, little is known about the impact of vascular immune cell subsets on atherosclerosis development and progression. To support the importance of the local vascular adaptive immune response, I will discuss the capacity of antigen presenting cells (APCs) to present *in vivo* systemically administered antigens directly into the vessel wall and will detail the kinetic of local vs systemic antigen presentation in experimental atherosclerosis. I then will focus on the role of the plasmacytoid dendritic cells (pDCs) (1), the main APC subset to expand in the mouse atherosclerotic aorta showing enhanced Ag presentation capacity. We first demonstrated that pDC depletion significantly reduced atherosclerosis formation in the aortic sinus leading to a more stable plaque phenotype (2). Subsequently, we identified a critical role for MHCII-restricted antigen presentation by pDCs in driving proatherogenic T cell immunity (3). Finally, I will discuss the impact of artery tertiary lymphoid organs (ATLOs) on disease progression. We found in aged apolipoprotein-E (apoe)$^{-/-}$ mice that ATLOs control aorta T cell responses. ATLOs promote T cell recruitment, prime CD4^{+} T cells, generate CD4^{+}, CD8^{+}, T regulatory (T_{reg}) effector and central memory cells. Vascular smooth muscle cell lymphotoxin β receptors (VSMC-LTβRs) maintain structure and size of ATLOs and protect against disease progression. In conclusion, vascular immune cells drive pro-atherogenic adaptive immunity in the early stages of the pathology; on the contrary, the immune system selectively employs ATLOs to organize vascular immunity and protect against plaque development during the advanced stages of the pathology.

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Pharmacogenomics And Immunomodulatory Effects Of Statins

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Statins have immunomodulatory and anti-inflammatory effects which can be seen by improved early outcomes and reduction of the inflammatory changes associated with acute coronary syndrome and improved survival in some infections, such as influenza. The immunomodulatory response to statins can affect T-cell subsets and this appears to benefit some inflammatory conditions such as rheumatoid arthritis. It has been suggested that the immunomodulatory effect of statins may increase the risk of cancer and increased incidence of certain cancers was seen in some individual studies with statins but overall the meta-analyses and long-term follow-up of the large statin studies have not shown any increased risk of cancers.

The anti-inflammatory effects, as measured by the reduction in hs-CRP, and the lipid modifying effects shown by the reduction in LDL-cholesterol both increase with the dose and the potency of statin treatment, but the changes in these two parameters are not well correlated in individuals. Some genetic determinants of the LDL-cholesterol response to statins have been identified. In the pharmacodynamic pathways, polymorphisms in the genes for apolipoprotein E and lipoprotein(a) have consistently been associated with LDL-cholesterol changes and some studies found that polymorphisms in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene also influenced the lipid response. In the pharmacokinetic pathways, the major effects on statin safety and efficacy are seen with polymorphisms in drug transporters. For simvastatin this involves the solute carrier organic anion transporter 1B1 (SLCO1B1) which influences liver uptake of simvastatin acid. Rosuvastatin pharmacokinetics are also influenced by SLCO1B1 but appear to depend to a greater extent on the efflux transporter ATP-binding cassette G2 (ABCG2). Changes in the inflammatory markers such as hs-CRP have not been associated with the polymorphisms influencing the pharmacokinetics and lipid responses suggesting the effects on these parameters diverge at an early stage in the different pathways.

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Targeting Inflammation To Reduce CVD Risk: A Realistic Clinical Prospect?

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Progression of atherosclerosis is the major pathophysiological process underlying ischaemic cardiovascular disease (CVD) such as myocardial infarction. No longer considered a passive degenerative disease, the role of the immune response in promoting atherosclerosis in the vessel wall has been a hot topic of investigation since the mid-1980s. The emerging evidence from basic science has stimulated debate as to whether we can successfully measure and/ or target inflammation to aid CVD prevention, giving rise to the following clinical questions: 1) Do inflammatory biomarkers, such as C-reactive protein, enhance prediction of CVD in a way that facilitates better targeting of existing preventative interventions, such as statin treatment?; 2) Are the protective effects of statins due partly to pleiotropic effects reducing systemic inflammation?; and, most importantly 3) Do anti-inflammatory medications offer safe and novel approaches to lessen CVD risk either in high risk groups, or in the general population, above and beyond established therapies? This presentation will critically assess evidence from observational epidemiological studies, mendelian randomization studies, and ongoing randomized controlled clinical trials to attempt to address these issues.

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Role of TRPC5 in Multidrug Resistance of Breast Cancer

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Tumor cells develop multidrug resistance during chemotherapy. Furthermore, drug resistance can be transferred from drug-resistance cancer cells to non-resistant cells via extracellular vesicles. However, the underlying mechanisms of chemoresistance and its intercellular transfer are not fully understood. An attractive strategy to overcome chemoresistance is to suppress P-glycoprotein (P-gp), which is overproduced in cancer cells to pump cytotoxic drugs from cells. In the present study, we found that the Ca^{2+}-permeable channel TRPC5 is overproduced together with P-gp in the adriamycin-resistant breast cancer cell line MCF-7/ADM. Suppressing TRPC5 activity/expression reduces the P-gp induction and causes a remarkable reversal of adriamycin resistance in these cells. In an athymic nude mouse model of adriamycin-resistant human breast tumor, suppressing TRPC5 decreases the growth of tumor xenografts. In addition, we found that adriamycin-resistant MCF-7/ADM cells possess numerous TRPC5-containing extracellular vesicles. Incubation of the TRPC5-containing extracellular vesicles with drug-sensitive wild-type MCF-7 cells results in P-gp overexpression in recipient cells and confers the drug resistance onto recipient cells. These findings yield several important conclusions. 1) TRPC5 is required for P-gp production via TRPC5-NFATc3-P-gp signaling cascade in drug-resistant cancer cells; 2) The property of drug-resistance can be transferred from drug-resistant cells to non-resistant cells via extracellular vesicle-mediated transfer of TRPC5.

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Protease-Biased Agonism of G Protein-Coupled Receptors and Transient Receptor Potential Ion Channels: Pathways to Pain and Neurogenic Inflammation

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Introduction. Proteases that are activated in disease can signal to cells by cleaving protease-activated receptors (PARs). Trypsin and tryptase cleave PAR_2 on primary sensory neurons at $R^{36}S^{37}$. Activated PAR_2 sensitizes transient receptor potential (TRP) channels, leading to neurogenic inflammation and pain. Whether proteases that cleave PAR_2 at alternate sites can activate distinct signaling pathways that also sensitize TRPs and cause neurogenic inflammation and pain is unclear.
Aims. To identify proteases that are activated in diseased tissues and determine whether these proteases are biased agonists of PAR_2 and TRP vanilloid 4 (TRPV4).
Methods. We used activity-based probes to identify proteases that are activated in inflamed tissues, and determined whether these proteases can cleave within the extracellular N-terminus of PAR_2. We examined the capacity of proteases to activate PAR_2 and TRPV4 in model cell lines, *Xenopus laevis* oocytes, and primary sensory neurons from mice. We determined whether proteases cause inflammation and mechanical hyperalgesia in mice by PAR_2- and TRPV4-mediated mechanisms.
Results. Macrophage cathepsin S and neutrophil elastase were activated in colon of mice and human subjects with inflammatory bowel disease. Cathepsin S cleaved PAR_2 at $G^{41}K^{42}$ and elastase cleaved PAR_2 at $A^{66}S^{67}$ and $S^{67}V^{68}$. Both proteases induced PAR_2 coupling to Gαs, formation of cAMP and activation of ERK1/2. In contrast to trypsin, these proteases did not induce PAR_2 coupling to Gαq, mobilization of intracellular Ca^{2+}, recruitment of β-arrestins or receptor endocytosis. Cathepsin S and elastase caused PAR_2-dependent sensitization and activation of TRPV4 in oocytes and primary sensory neurons by adenylyl cyclase- and protein kinase A-dependent mechanisms. Intraplantar injection of cathepsin S or elastase evoked inflammatory edema and mechanical hyperalgesia, which were diminished in mice lacking PAR_2 or TRPV4.
Discussion. Proteases that cleave at unique sites can act as biased agonists of PAR_2 and TRPV4. Thus, PAR_2 is a molecular integrator of the actions of diverse proteases.

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Ion Channels and Cold Allodynia

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Introduction. Cold-induced pain is a frequent symptom in neuropathic pain including chemotherapy-induced neuropathy, and a hallmark feature of the fish poisoning disease ciguatera. Cold temperature detection involves the process of sensory transduction in the cutaneous primary sensory nerve terminals, which converts a thermal stimulus into depolarisation of the membrane. This transformation into an electrical signal is followed by the subsequent propagation of the action potentials in the cold-sensitive afferent nerves. A large array of ion channels, including TRPs and sodium and potassium channels, shape this process. However, compared to other pain modalities, such as heat pain, the precise role of specific ion channel subtypes in cold pain and allodynia remain elusive.
Aims. The aim of this work was to elucidate the relative contributions of different ion channel types and isoforms to cold allodynia, hyperalgesia and cold sensing to define the molecular mechanisms underlying cold pain symptoms in disease.
Methods. We assessed the mechanisms underlying ciguatoxin- and oxaliplatin-induced cold allodynia using *in vivo* behavioural characterisation, Ca^{2+} imaging of cultured sensory neurons, and extracellular recordings from the murine skin – saphenous nerve preparation.
Results. We showed that ciguatoxin-induced cold allodynia relies on $Na_v1.8$-expressing sensory neurons and the presence of the cold sensor TRPA1. In contrast, oxaliplatin-induced cold allodynia develops through inhibition of potassium channels in $Na_v1.6$-positive myelinated fibres.
Discussion. These findings highlight the complex, disease-and pathway-specific roles of ion channels in pathological pain states and identify novel treatment approaches for pain.

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New Approaches To Targeting Calcium Channels In Breast Cancer

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Intracellular calcium signalling is a key regulator of a number of cellular processes relevant to the hallmarks of cancer, including proliferation and invasion. The remodelling of specific aspects of calcium signalling is a feature of some breast cancer cells. Significant alterations in the expression levels of some calcium channels are a characterizing feature of specific breast cancer subtypes, including those associated with poor prognosis, such as the basal breast cancer molecular subtype. Exploration of the potential therapeutic targeting of calcium channels in breast cancer has predominately focused on pharmacological inhibition of an overexpressed calcium channel in order to reduce breast cancer cell proliferation or migration. This presentation will describe alternative approaches for the identification and targeting of calcium channels as therapeutic targets in breast cancer. Such approaches include the characterisation of calcium dependent processes important in events promoting breast cancer metastasis such as hypoxia and epidermal growth factor-mediated epithelial to mesenchymal transition. Such studies have characterised the remodelling of calcium influx associated with epithelial to mesenchymal transition and have identified specific calcium permeable ion channels that are critically important for the initiation of epithelial to mesenchymal transition in MDA-MB-468 breast cancer cells. The presentation will also describe alternative pharmacological approaches to targeting overexpressed calcium channels that promote breast cancer cell death.

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The Association Between CYP3A4 Genetic Polymorphism And The Susceptibility Of Breast Cancer In Chinese Han Female Population

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Introduction. Estrogen plays a key Role in breast cancer development and functionally relevant genetic variants within the estrogen metabolic pathway are prime candidates for a possible association with breast cancer risk.
Aims. To explore the association between CYP3A4 genetic polymorphisms and the susceptibility of breast cancer.

Table 1 Analysis of relationship between genotype and alleles of CYP3A4*1G and the susceptibility of breast cancer

Type	Cases(%)	Controls(%)	P#	OR(95%CI)
*1/*1	71.6	56.9	<0.01	0.33-0.85
*1/*1G+ 1G/*1G	28.4	43.1		
*1	84.1	77.8	<0.05	0.44-0.98
*1G	15.9	22.2		

Methods. A case-control study was carried out to compare the distribution of genotypes and genetic frequencies of CYP3A4*1G between breast cancer (n=148) and healthy volunteers (n=160) with polymerase chain reaction-direct sequencing method.
Results. The distribution of genotypes and genetic frequencies of CYP3A4*1G were both in accordance with Hardy-Weinberg balances (P>0.05). The frequencies of CYP3A4*1G allele in the cases and controls were 15.9% and 22.2%, respectively (P<0.05). There was also significant difference of CYP3A4*1G genotypes between the cases and controls (P<0.01).
Conclusion. The *1G allele of CYP3A4 was inversely correlated with the susceptibility of breast cancer.
Declaration.This work that has not been published or presented at another national meeting.

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A Vital Role of Drug Binding Kinetics on Biased Agonism at GPCRs

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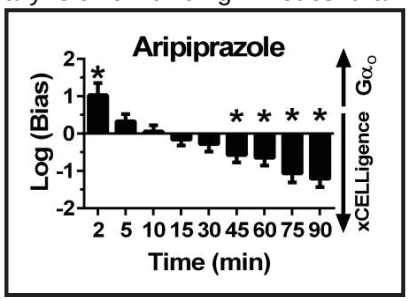
Introduction. Biased agonism reflects the ability of different ligands to stabilize distinct conformations of a given G protein-coupled receptor (GPCR) each linked to distinct functional outcomes. A GPCR at which biased agonism has been extensively studied is the dopamine D_2 receptor (D_2R). However, in all studies to date, a conformational mechanism of bias is inferred from pharmacological data with the assumption that the confounding influences of observational (i.e., assay-dependent) and system (i.e., cell background-dependent) bias are excluded by experimental design and analysis.
Aim. To determine if ligand-binding kinetics can influence observations of biased agonism.
Methods. Binding experiments were performed to determine the binding kinetics of D_2R agonists. The effects of D_2R agonists were measured at multiple signalling pathways at various time-points. Agonist effect and biased signalling at each timepoint was quantified using an operational model of agonism (1).
Results. Cariprazine, aripiprazole and bifeprunox displayed substantially slower binding kinetics than other agonists, including dopamine. Ligands with slower binding kinetics displayed higher potency with increasing time, consistent with an increase in receptor occupancy. However, the relative change in potency was pathway dependent. This resulted in a significant change and even reversal of the bias profile over time.



Discussion. This study indicates that the influence of ligand binding kinetics should be considered in future studies of biased agonism and has major implications for the current interpretation of biased agonism at the D_2R and other GPCRs.

(1) Kenakin T. et al. (2012) ACS Chem. Neurosci. 3:193-203

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A Novel UPLC-MS Approach For The Quantification Of 13 Clinically Used Kinase Inhibitors

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Introduction. Kinase inhibitors (KIs) are a rapidly expanding class of anticancer drugs that have achieved remarkable success in extending the survival of cancer patients. Most clinically used KIs are cleared by metabolism, and thus there is substantial potential for intra-individual variability in drug exposure. This variability is inadequately addressed by fixed protocol dosing resulting in suboptimal use and potential therapeutic failure or toxicity. One way to address this issue is with individualised dosing based on plasma KI concentration; however, there exists a need to facilitate this process. Currently it is not possible to predict which patients are likely to reach plasma concentrations within a target range, therefore a need for therapeutic drug monitoring (TDM) exists to identify optimal dosing for each individual. This approach may maximise the effectiveness of KIs while minimising toxicity.
Aim. Establish a panel-based analytical approach for the quantification of 13 TKIs currently used at Flinders Centre for Innovation in Cancer to facilitate dose optimisation in clinical practice.
Method. Human plasma was spiked with KIs. Analytes were isolated by solvent extraction and separated by ultra-performance liquid chromatography using a 150mm x 2.1µm T3 HSS column and a mobile phase comprising 10mM ammonium formate with an acetonitrile gradient (10-90%). Analytes were detected by quadrupole time-of-flight tandem mass spectrometry using positive electrospray ionisation mode.
Results. Linear calibration curves were created over the range of 10-5000µg/L for gefitinib, imatinib, nilotinib and sorafenib, 10-1000µg/L for dabrafenib, dasatinib, erlotinib, lapatinib, vemurafenib, sunitinib active and pazopanib and 0.1-250µg/L for sunitinib and 10-500µg/L for axitinib. Peaks for each analyte were adequately separated. Extraction recovery was in the range of 85-110%. Intra-and inter-day precision was fit for purpose with coefficients of variation <10%. LOQ ranged from 0.331-5.6µg/L.
Discussion. This novel comprehensive assay can quantify 13 KIs, facilitating the need to perform TDM in clinical care for individualised dosing guided by plasma KI concentrations.

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Potential Therapeutics to Prevent Vision Loss Associated with Retinal Neovascularisation by Targeting NADPH Oxidase Dusting GJ, Chan EC, van Wijngaarden P, Hakami N, Peshavariya HM
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Introduction. We and others have shown that NADPH oxidase (Nox) is crucial for cell signaling leading to aberrant neovascularization of the retina. There is an urgent need for easily administered therapeutics to manage this growing cause of blindness associated with macular degeneration and diabetic retinopathy.
Aim. To determine whether a synthetic flavonol prevents retinal neovascularization via NADPH oxidase.
Methods. In human endothelial cells Nox isoforms and adhesion molecule expressions were evaluated by real time PCR. Retinal neovascularisation was induced by exposing neonatal wildtype and Nox4$^{-/-}$ mice aged 7d (P7) to 5d of hyperoxia (75%O_2) followed by room air. Mouse pups' eyes were harvested on P8 and P17 for quantitation of retinal vaso-obliteration and neovascularization, respectively, as described (1).
Results. Suppression of Nox4 *in vitro* using either RNAi or a synthetic flavonol (2HF, 10-50μM, shown to inhibit Nox4 expression) markedly reduced endothelial proliferation and tubulogenesis. 2HF also abolished cytokine-induced ICAM-1 and VCAM-1 expression. In wild-type mice, oxygen-induced retinopathy was associated with intense neovascularization at P17, and increased expression of both Nox4 (12-fold) and Nox2 (4-fold) as well as VEGF (2.5-fold). Nox4$^{-/-}$ mice exhibited markedly less retinal neovascularization (7.9±0.7%, n=13) but similar elevations of VEGF (2-fold) as wildtype (13.6±0.8% and 2.5-fold, respectively; n=14). VEGF did not increase in Nox2$^{-/-}$ mice. Like Nox4$^{-/-}$, daily treatment with 2HF (1 mg/kg, ip) reduced retinal neovascularization (by 30%) compared to vehicle-treated, wildtype controls.
Discussion. Clearly Nox4 facilitates retinal neovascularization in these mice. Drugs such as specific flavonols that target Nox4 could be of therapeutic value to reduce aberrant retinal neovascularization in retinopathy associated with prematurity. 2HF also has therapeutic potential for the coming epidemics in the elderly suffering vision loss from diabetes or proliferative macular degeneration.

(1) Chan EC et al (2013) *Invest Ophth Vis Sci* **54**: 7061-7067.

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Post-approval Withdrawal Of Medicinal Products Because Of Adverse Drug Reactions: A Systematic Review Of The World Literature
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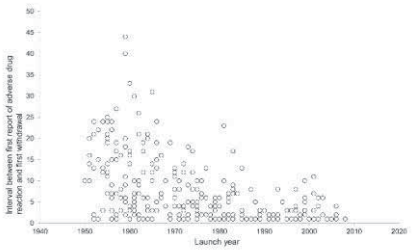

Introduction. There has been no comprehensive examination of the pattern of drug withdrawals because of adverse reactions across countries and the evidence that influences such decisions.
Aims. To identify medicinal products that have been withdrawn because of adverse drug reactions after regulatory approval, to examine the evidence used for such withdrawals, and to explore the pattern of withdrawals across countries.
Methods. We searched Pubmed, GoogleScholar, the WHO's database of drugs, the websites of drug regulatory authorities, and textbooks. The level of evidence used for making withdrawal decisions was assessed using standard criteria [http://www.cebm.net/index.aspx?o=5653].
Results. We identified 428 medicinal products that were withdrawn between 1953 and 2011. The evidence used for withdrawal in over 70% of cases consisted of anecdotal reports. Withdrawals were significantly more common in Europe than in Africa ($P < 0.0001$). The interval between the year of first launch and the year of first withdrawal has consistently shortened over time. However, the interval between the first report of the adverse drug reaction that led to withdrawal and the subsequent first withdrawal has not consistently shortened (Figure).
Discussion. There are no universally accepted guidelines for determining when a drug should be withdrawn. Regulatory authorities and drug manufacturers should expedite action when adverse drug reactions are suspected. Drug monitoring systems in low- to middle-income economies should be strengthened. Greater transparency in the reporting of adverse events in clinical trials would be beneficial.

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Computational Insight into The Mechanism For The Metabolic Activation Of N'-nitrosonornicotine

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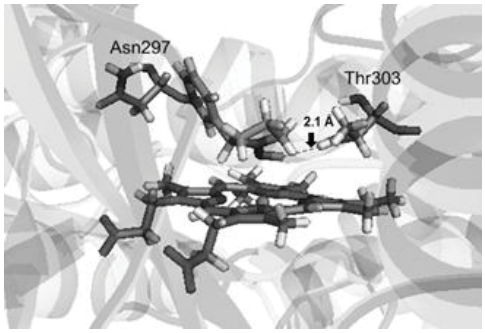

Introduction. N'-nitrosonornicotine (NNN) is a kind of tobacco-specific nitrosamine (TSNA). Studies have shown that malignant tumors in many tissues can be induced by NNN in rodent animals and humans (1). Both of the metabolic reaction on the 2' and 5' positions of NNN can be catalyzed by cytochrome P450 (CYP450). There are two stereoisomers, (R)-NNN and (S)-NNN, due to the charity of 2'-C_{α}.

Aims. The purpose of the study is to investigate the mechanism of the metabolic activation and the preferential pathway of NNN.

Methods. Our study using Gaussian 09 Density Functional Theory (DFT) calculations on the B3LYP/LANL2DZ (Fe)/6-31+G (d, p) (H,C,N,O,S) level to calculate the H-abstraction process from the 2'-and 5'-C of (R)-NNN and (S)-NNN and obtained the single-point energy at the LANL2DZ (Fe)/6-311++G (2df, p) (H,C,N,O,S) level. We also investigate the interaction of NNN with various subtype of CYP450 by molecular docking in GOLD program.

Results.The results showed that the metabolic reaction on the 2'-position is the preferential pathway of NNN. The docking result indicated that the 2'-position of (S)-NNN was more easily to be metabolized by CYP450. The CYP450 2A6 in figure has the best conformation and highest score.

Discussion.The study suggested that the interaction of CYP450 enzyme with NNN substrate was the key factor in determining the organ specificity in the carcinogenesis of NNN.

(1) Hecht SS (1998) Chem Res Toxicol **11**: 559-603.

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Decrease In Blood Inorganic Mercury Level But Increase In Blood Organic Mercury Level In The American Population 2005-2012

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Introduction: Mercury is in the environment and is concentrated in fish. We previously reported decreasing blood inorganic mercury level but a possible increase in blood organic mercury level in US.

Aim: We sought to confirm these trends using the latest data available.

Methods: Blood levels of total, inorganic and organic mercury (geometric mean [95% CI]) in the US National Health and Nutrition Examination Survey (NHANES) 2005-2012 were analysed using the complex sampling function of SPSS version 22 (IBM). Organic mercury level was calculated by subtracting inorganic from total mercury level. Sample weights were used to adjust for sampling bias.

Results:	2005/6	2007/8	2009/10	2011/12
Number of participants	8364	8161	8727	7920
Total Hg, µg/L	0.87 [0.79-0.95]	0.78 [0.70-0.88]	0.87 [0.79-0.94]	0.70 [0.62-0.80]
% with total Hg>5.8 µg/L	3.0±0.2	3.5±0.6	4.0±0.4	3.2±0.7
Inorganic Hg, µg/L	0.31 [0.30-0.32]	0.30 [0.30-0.31]	0.28 [0.27-0.28]	0.23 [0.23-0.24]*
Organic Hg, µg/L	0.24 [0.19-0.30]	0.19 [0.14-0.25]	0.27 [0.22-0.33]	0.39 [0.30-0.51]*
Organic Hg, µg/L, age<20	0.05 [0.04-0.06]	0.04 [0.03-0.05]	0.05 [0.04-0.07]	0.11 [0.09-0.14]*
Organic Hg, µg/L, pregnant	0.13 [0.07-0.23]	0.20 [0.11-0.35]	0.25 [0.14-0.47]	0.35 [0.21-0.59]*

*P<0.05 for trend

Discussion: Blood inorganic mercury level steadily decreased. Although only a small proportion of people had a blood mercury level above safe levels (>5.8 µg/L), blood organic mercury level has increased recently in the general population, children and pregnant women. Monitoring mercury levels in man and environment should continue, and so should measures to reduce exposure to mercury.

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Identification of amyloid forming regions and their cell toxicity of the HIV infection-promoting amyloid peptide SEVI.

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Extracellular deposition of highly structured protein aggregates termed amyloid fibrils are involved in several debilitating and incurable human diseases including Alzheimer's disease, Parkinson's disease, systemic amyloidosis and type 2 diabetes. Amyloid fibrils arise when a specific protein or protein fragment loses their correct folding status, aggregate and form insoluble deposits. The precipitating factors can be due to stresses like elevated temperature, low pH, infection, reactive oxygen species and inherited mutations. Semen contains a fibril forming peptide that significantly increases the ability of HIV to infect the cells by helping the virus to attach to the cells. The peptide is a fragment of prostatic acidic phosphatase and its fibrils are termed Semen-derived enhancer of virus infection (SEVI). The aims of this study were 1) to identify potential amyloidogenic regions in SEVI and determine whether these putative amyloidogenic regions are able to form amyloid fibrils and 2) to determine whether SEVI and its fragments were toxic to neuronal PC 12 cells, a standard model for the toxicity of amyloid β (Aβ, the putative causative agent in Alzheimer's disease), and human epithelial CACO-2 cells as a model of epithelial cells. Our results determined the fibril forming regions of SEVI. The results of this study also found that, similar to beta amyloidm SEVI and its fibril forming fragments are toxic to neuronal cells. However SEVI, again like beta amyloid, was not toxic to confluent epithelial cells. These findings imply that although SEVI assists the attachment of HIV-1 to immune cells, it does not facilitate HIV entry by compromising the epithelial cell layer that presents a barrier to HIV. Importantly, the presence of SEVI fragments in semen could also promote HIV infection along with SEVI itself.

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When Good Guys Turn Bad

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The endothelial cells control the tone of the underlying vascular smooth muscle by releasing the vasodilator substances prostacyclin and nitric oxide (NO). However, under pathological conditions those two beneficial mediators can elicit endothelium-dependent contractions. The latter are exacerbated by aging, diabetes and hypertension. In most cases this exacerbation is due to the exaggerated release of prostacyclin which activates thromboxane/prostaglandin (TP) receptors of the vascular smooth muscle cells, causing their contraction. Thus, prostacyclin can become an endothelium-derived contracting factor. The same is true for NO. Indeed, in coronary arteries, hypoxia causes an acute augmentation of vasoconstrictor responses that is dependent on the presence of NO and activation of soluble guanylyl cyclase. This hypoxic effect is due to increases in the intracellular level of inosine 5'-triphosphate (ITP) and the biased activity of soluble guanylyl cyclase which results in the synthesis of inosine 3',5'-cyclic monophosphate (cIMP). Similar endothelium-dependent, NO-dependent and soluble guanylyl cyclase-dependent contractions can be evoked with thymoquinone, which also augments the levels of cIMP. The understanding of the role of this non-canonical cyclic nucleotide may help identifying novel therapeutic targets for certain cardiovascular disorders, in particular those associated with sleep apnea.