ASCEPT-BPS

JOINT SCIENTIFIC MEETING

Tomorrow's medicines: pharmacology, patients and populations

19-21 May 2015

University of Hong Kong

POSTER ABSTRACTS

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Poster Programme

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414	An In Vitro Study Of Compounds With Selectivity For Human Neuronal Sodium Channel Subtypes	Bevyn Jarrott	Molecular and Cellular pharmacology
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418	Osteopontin Suppresses FcεRI Activation of Human Mast Cells by Inhibiting Chemokine, Th1 and Th2 Cytokines Releases	Chun Wai Ng	Molecular and Cellular pharmacology
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504	Exogenous Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) Pathway Increases Atherogenesis	Wen Cheng	Cardiovascular and Respiratory
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506	Investigation Of Directly And Indirectly Mediated Cardiovascular Actions Of Stimulants	James Docherty	Cardiovascular and Respiratory
507	Deletion Of SIRT1 In Perivascular Adipose Tissue Accelerates Obesity-Induced Endothelial Dysfunction	Hannah Hui	Cardiovascular and Respiratory
508	Effects Of Isoflavones On The Release Of Inflammatory Mediators By Cigarette Smoke In Airway Epithelial Cells	Susan Leung	Cardiovascular and Respiratory
509	Investigation Of The Antiarrhythmic Mechanism Of The Multi-Herbal Medicine Xing Su Ning	Yu-ling Ma	Cardiovascular and Respiratory
510	Effects Of Taurine On Contractions Of Human Internal Mammary Artery	Melik Seyrek	Cardiovascular and Respiratory
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513	Pharmacological Inhibition Of Protein Tyrosine Phosphatase 1B Promotes Endothelial Cells On Cell Spreading And Migration	Yuan Wang	Cardiovascular and Respiratory
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519	Novel 5-HT3 Receptor Antagonist (4-Phenylpiperazin-1-Yl) (Quinoxalin-2-Yl) Methanone (4a) Reverses The Altered Plasma Corticosterone, Leptin And Brain Oxidative Stress In Depression Co-Morbid With Obesity In Mice	Yeshwant Kurhe	Neuropharmacology
520	Neuroprotective Action Of GSCF And Dizocilpine In Cerebral Ischemia Induced Neuronal Injury	Bikash Medhi	Neuropharmacology
521	Tianeptine Differentially Affects Oxidative Phosphorylation and Neuroprotection Compared with Other Antidepressants	Michael Spedding	Neuropharmacology
522	Depolarisation-Induced Gene Changes In Isolated Dorsal Root Ganglion Cells	Kai-Hei Tse	Neuropharmacology
523	No Evidence Of A Role For Renal Eicosanoid-Producing Cytochrome P450 Enzymes In Cadmium-Linked Kidney Disease	Kanyarat Boonprasert	Toxicology

Thursday 21 May 2015 10:30 - 11:00 and 13:00 - 14:00

Abstract number Board number	Speakers Paper Title	Presenter	Speakers Theme Description
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601	Influence Of CYP3A Polymorphisms On The Pharmacokinetics Of Oral Midazolam And The Urinary 6β-Hydroxycortisol/Cortisol Ratio As Markers Of CYP3A Activity In Healthy Male Chinese	Sze Wa Chan	Drug Discovery, Development and Evaluation
602	Anti-oxidative Efficacy Of Crude Water Extract Of Mangosteen Peel In Alzheimer Patients	Nattapon Jaisupa	Drug Discovery, Development and Evaluation
603	Effects Of General Practitioner Education On The Prescription Of Anticholinergic Medications	Karen Kerr	Drug Discovery, Development and Evaluation
604	The Protein Kinase Inhibitors Trametinib And Dabrafenib Inhibit Human UDP-Glucuronosyltransferase 1A4 And 1A9	Porntipa Korprasertthaworn	Drug Discovery, Development and Evaluation
605	Polypharmacy, Age-dependent Physiological Changes And Risk Of Falls In Older People	Snezana Kusljic	Drug Discovery, Development and Evaluation
606	Protective Effects Of CHA79, A Synthetic Chalcone Derivative, On Methylglyoxal-Induced Neurotoxicity	Yi-Ching Lo	Drug Discovery, Development and Evaluation
607	Application Of SPECT/CT Imaging System For Investigation Of Blood Kinetics And Tissue Distribution Of Radiolabeled Plumbagin In Healthy And Plasmodium Berghei-Infected Mice	Kesara Na-Bangchang	Drug Discovery, Development and Evaluation
608	Evaluation Of Modafinil As A Perpetrator Of Clinically Relevant Metabolic Drug-Drug Interactions	Angela Rowland	Drug Discovery, Development and Evaluation
609	Targeting Arginine Metabolomics In Older Individuals	Andrew Rowland	Drug Discovery, Development and Evaluation
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611	Information Seeking Behaviors Of Breastfeeding Women When Considering The Use Of Over-The-Counter Medicines	Alison Shield	Drug Discovery, Development and Evaluation
612	A Study Of The Effects Of Andrographis Paniculata On Platelet Activity In Thai Healthy Volunteers: The Pilot Study	Tichapa Sirikarin	Drug Discovery, Development and Evaluation
613	An Exploratory Study Of The Dietary Patterns Of North East Asian Subjects Participating In An Australian Phase I Clinical Study	Sophie Stocker	Drug Discovery, Development and Evaluation
614	Metformin Suppresses The Pro-Metastatic Protein, CXCR4, Through HER2 Inhibition	Benny Tan	Drug Discovery, Development and Evaluation
615	Deprescribing In Australian Residential Aged Care Facilities: What Do GPs, Nurses, Pharmacists And Residents Consider Important?	Simon Bell	Drug Discovery, Development and Evaluation
616	What Factors Do Multidisciplinary Care Teams Consider Important When Deprescribing In Australian Residential Aged Care Facilities?	Simon Bell	Drug Discovery, Development and Evaluation
617	Two Blood Test Parameters, RDW And AST May Predict High Serum Valproic Acid Levels In Epileptic Patients	Kemal Gökhan Ulusoy	Drug Discovery, Development and Evaluation
618	Nrf2-mediated Inhibition Of Neuroinflammation By Tiliroside	Ravikanth Velagapudi	Drug Discovery, Development and Evaluation
619	Determination of Aristolochic Acid I in Ayurved Siriraj Ammareutavatee Recipe Using Solid Phase Extraction And HPLC	Jantanee Wattanarangsan	Drug Discovery, Development and Evaluation
620	KMUP-1 Reduces Neuropathic Pain Via PKA And PKC Signaling In Dorsal Root Ganglion	Bin-Nan Wu	Drug Discovery, Development and Evaluation
621	MicroRNAs As Biomarkers For Alzheimer's Disease—A Systematic Review And Meta-analysis	Helen Wu	Drug Discovery, Development and Evaluation

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Mechanical Characteristics Of Isolated Middle Cerebral Arteries Of Newborn Piglets – Does Age Matter?

Vibeke R Eriksen[1,2], Simon Trautner[2], Gorm Greisen[2], Majid Sheykhzade[1]. Dept of Drug Design and Pharmacology, Faculty of Health Science, University of Copenhagen[1], Copenhagen, Denmark; Dept of Neonatology, Rigshospitalet[2], Copenhagen, Denmark.

Introduction. Premature and mature newborn piglets are often used as an animal model to study regulation of cerebral blood flow in newborn infants – a mechanism maintained by cerebral arteries. However, mechanical characteristics of newborn piglets' cerebral arteries are not well defined.
Aims. To compare passive and active mechanical properties of cerebral arteries from premature and mature piglets. Our hypothesis is that they differ due to structural and functional immaturity.
Methods. Premature piglets born at 90% gestational age were euthanized on day 5 (N=6) or day 26 (N=3). Mature piglets were euthanized on day 1 (N=6). 2nd order middle cerebral arteries were isolated and mounted on wire myographs. Passive and active properties of the segments in these three different age-groups were compared using ANOVA.

Parameters	Premature 5 days	Premature 26 days	Mature 1 day	p-value
$n_{segments}$	14	5	19	
Normalised diameter (µm)	307±15	311±25	367±32	0.255
AWT_0 (Nm^{-1})	1.16±0.16	1.22±0.23	1.16±0.22	0.990
PWT_0 (Nm^{-1})	1.32±0.13	1.32±0.23	1.72±0.20	0.230
IC_0/IC_{100}	0.84±0.03	0.85±0.05	0.93±0.10	0.701

Results. IC_0 represents the internal circumference where maximal active wall tension (AWT_0) was developed. PWT_0 is passive wall tension at IC_0. IC_{100} is the internal circumference of MCAs at a simulated passive transmural pressure of 100 mmHg.
Discussion. Passive and active properties of 2nd order middle cerebral arteries did not differ among premature piglets and piglets born at term. The ratio IC_0/IC_{100} was comparable with previously shown ratios from rat mesenteric small arteries. Our conclusion is that gestational age and postnatal age do not appear to affect the active and passive mechanical properties of pial arteries in neonatal piglets.

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Evaluating a Physiologically-Based Pharmacokinetic Model for Prediction of Pharmacokinetics of Omeprazole in Chinese

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Introduction. Physiologically-based pharmacokinetic (PBPK) model can be a useful tool to simulate pharmacokinetics in various ethnic groups and assess ethnic sensitivity.
Aims. The objective is to evaluate the ethnicity-specific population models in the SimCYP Simulator® for prediction of omeprazole PK with attention to differences in the CYP2C19 metabolic pathway.
Methods. The SimCYP Simulator® models incorporating Chinese, Caucasian and Japanese population specific demographic, physiological and enzyme data were applied to simulate omeprazole pharmacokinetics. Published PK data of omeprazole after intravenous or oral administration in Chinese were used for the evaluation.
Results.Significant differences (2-fold) in the observed oral clearance of omeprazole were identified between Caucasians and Asian (Chinese and Japan) while the observed oral and intravenous clearances of omeprazole were similar between Chinese and Japanese.
Discussion. The PBPK model within SimCYP adequately predicted pharmacokinetics of omeprazole in Chinese and the 2-fold differences in clearance of omeprazole between Caucasians and Asian (Chinese and Japanese). This may lead to early identification of ethnic sensitivity in PK for substrates of CYP2C19.

(1) ZE Barter (2013) Clin Pharmacokinet. 2013 Jul 2
(2) Wang Y et al (2010) Eur J Clin Pharmacol. 66(6):563-9
(3) Yin OQ et al (2004) J Clin Pharmacol. 44(6):582-9

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Addition of Berberine to 5-ASA in Dextran Sulfate Sodium-Induced Ulcerative Colitis in C57BL/6 Mice: Efficacy, Mechanism-of-Action, and Safety

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Introduction: Ulcerative colitis (UC) is a very common inflammatory bowel disease (IBD) but without satisfactory treatment. Alternative medicine berberine has received massive attention for its potential in the treatment of UC. Conventional therapies with the addition of berberine are becoming attractive as novel therapies in UC.
Aims: In the present study, we investigated the preclinical activity of a conventional oral 5-aminosalicylic acid (5-ASA) therapy with the addition of berberine in experimental colitis.
Methods: A subclinical dose of 5-ASA (200 mg/kg) alone or 5-ASA combined with berberine (20 mg/kg) was administered orally via gavage for 30 days to C57BL/6 mice with UC induced by three cycles of 2% dextran sulfate sodium (DSS). Clinical parameters, intestinal integrity, proinflammatory cytokines, STAT-3 signalling, and potential toxicity were examined.
Results: Results showed that comparing with 5-ASA alone, 5-ASA combined with berberine more potently ameliorated DSS-induced body weight loss, shortening of the colon, injury and inflammation scores, and reduction of colonic expression of tight junction (TJ) protein ZO-1 and occludin. Further, the upregulated proinflammatory cytokines and activated STAT3 in the colon caused by DSS were more significantly reversed in animals treated with the combination therapy than those treated with 5-ASA alone. H&E staining of the major organs of animals did not detect any increased toxicity in the combination group when compared to 5-ASA alone treatment.
Discussion: In summary, our studies provide preclinical rationale for addition of berberine to 5-ASA as a promising therapeutic strategy in clinic.
Acknowledgment: This work was supported by grants from the Food and Health Bureau of The Government of HKSAR [Health and Medical Research Fund (HMRF)10111971].

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Optimisation of Cocktail Drug Interaction Study Design Using PB-PK Simulations

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Introduction. MODDI-14 is a cocktail drug interaction study to assess modafinil as a potential reversible or irreversible inhibitor and/or inducer of drug metabolising cytochrome P450 enzymes (CYP). Subjects are orally administered the Inje cocktail comprising the selective CYP probes caffeine (CYP1A2), losartan (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6) and midazolam (CYP3A). The pharmacokinetics of probes is determined prior to modafinil exposure, following a single dose of modafinil (200 mg PO) and following steady-state dosing of modafinil. Although clinical drug interaction studies are routinely used to assess new drug candidates, the impact of single dose versus steady-state dosing on CYP activity is not usually investigated.
Aim. To optimise a cocktail drug interaction study design for the assessment of all potential changes in CYP activity by modafinil.
Methods. Physiologically-based pharmacokinetic (PB-PK) simulations were undertaken in healthy subjects (10 trials of 12 subjects each) using validated profiles for CYP probes and modafinil in Simcyp®. The minimal time to repeat dosing with CYP probes was defined by residual plasma probe concentrations of < 5%. The minimal dosing duration required for modafinil to achieve steady-state was determined on the basis of repeat trough concentrations within 5%.
Results. PB-PK simulations demonstrated that modafinil dosing is required for a minimum of 7 days to give a reproducible steady-state trough concentration. The optimal Inje cocktail regimen was achieved with CYP probes administered at t = 0 hr (prior to modafinil exposure), at t = 48 hr (following a single dose of modafinil at t = 47 hr), and at t = 192 hr (following daily dosing of modafinil for 7 days).
Discussion. An optimised cocktail drug interaction study design was determined using PB-PK simulations. This design is currently being used in the MODDI-14 study.

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Impact Of α-Adrenoceptor Antagonists On Biochemical Relapse In Men Undergoing Radiotherapy For Localised Prostate Cancer

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Introduction: Radiotherapy is a major part of the curative and palliative management of prostate cancer. Many men diagnosed with prostate cancer use α-adrenoceptor antagonists (α-AA) to control symptoms of the disease prior to radiotherapy and in-vitro studies suggest these medications may improve patient outcomes by enhancing the effect of radiotherapy.
Aims: To determine the impact of α-AA treatments on time to biochemical relapse following radiotherapy.
Methods: A retrospective study of male patients receiving radiotherapy for biopsy-proven localised prostate cancer was undertaken to compare cancer outcomes for drug-naïve patients and those receiving α-AA treatment. Ethical approval for the collection of data at Genesis CancerCare QLD was obtained and biochemical relapse (defined by a PSA rise of >2ng/mL above the nadir) was recorded in months. Treatment groups were those receiving α-AA treatment prior to or concurrent with their radiotherapy. Data was statistically analysed and expressed as mean±SD.
Results: The mean time to biochemical relapse for tamsulosin, prazosin, alfuzosin and controls were 45.3±17.4 (n=36), 41.5±19.6 (n=11), 29.3±6.02 (n=6) and 36.5±17.6 (n=16) months respectively. Tamsulosin, prazosin but not alfuzosin delayed time to biochemical relapse although the differences were not statistically significant (one-way ANOVA).
Discussion: Preliminary data for the prior and/or concurrent use of tamsulosin and prazosin showed a positive trend in delaying time to biochemical relapse although no statistical significance was shown. Larger clinical studies are indicated and with thousands of patient records yet to be analysed, it may determine if there is significant effect of these drugs on control of prostate cancer.

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Effect of Intravitreal Endothelin-1 on Retinal Morphology and RGC Survival

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Introduction. Elevated endothelin-1 (ET1) levels are associated with loss of retinal ganglion cells (RGCs) in glaucoma. ET1, a potent vasoconstrictor, causes retinal ischemia resulting in RGC loss. Experimental studies have used intravitreal ET1 in varying doses to investigate mechanisms involved in ET1-induced RGC loss and to investigate experimental neuroprotective drugs. However, the effect of ET1 on retinal morphology and RGC survival with changes in the dose remains unclear.
Aims. To evaluate the dose-dependent effects of ET1 on retinal morphology and RGC survival using retinal morphometric measurements.
Methods. 150-200g Sprague Dawley rats were divided into 4 groups (n=4). Groups 1, 2, 3 and 4 were intravitreally administered with vehicle, ET1 500 nmol, ET1 50 nmol and 5 nmol, respectively. Seven days after ET injection, rats were sacrificed and eyes were enucleated for H&E staining. Subsequently, retinal morphometric measurements were done.
Results. Retinal morphometry showed that volume density (%) of RGCs in ganglion cell layer (GCL) was 1.71, 1.45 and 1.33 folds lower in groups 2, 3 and 4, respectively, compared to group 1. The volume density (%) of RGCs in inner retina was 1.53, 1.38 and 1.15 folds lower in groups 2, 3 and 4, respectively, compared to group 1. The linear density of RGCs in GCL (cells per µm) was 2.07 ($p=0.01$), 1.81 ($p<0.05$) and 1.67 ($p<0.05$) folds lower in groups 2, 3 and 4, respectively, compared to group 1.
Conclusion. Intravitreally injected ET1 affects retinal morphology and reduces RGC survival in a dose - dependent manner.
We acknowledge financial support under grant no. 600-RMI/RAGS 5/3 (40/2014) by MOE, Malaysia.

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Molecular Determinants Of Selective Ligand Binding At The Human Melatonin Receptor

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Introduction. We have recently identified a series of substituted isoquinolinones (1) that act as highly selective agonists for the melatonin MT_2 receptor. These ligands represent valuable tools for delineating potential structural determinants that underlie the molecular properties of the MT_1 and MT_2 receptors.
Aims. To identify the key residues located at TM5, TM6 and TM7 of the melatonin receptors that contribute to the isoquinolinone binding pocket and govern subtype selectivity.
Methods. Conserved residues on both MT_1 and MT_2 receptors, including those which had been reported to have essential interactions with melatonin, were subjected to Alanine substitution. Selection of residues was guided by molecular modelling of the $MT_{1/2}$ receptors. Wild-type and mutant receptors were examined for Ca^{2+} mobilization upon isoquinolinone stimulation in transfected cells. Expression of receptor mutants was confirmed by radioligand binding assays.
Results. Mutation of an essential MT_2 residue, His208, severely impaired the potency of melatonin but did not affect the isoquinolinones. Mutation of two conserved tyrosine residues on TM7 to alanine (MT_1 Tyr281/Tyr285 and MT_2 Tyr294/Tyr298, respectively) had no effect on melatonin. In contrast, the MT_2-selective isoquinolinones lost their selectivity and became able to activate the MT_1 mutant receptor.
Discussion. Our mutagenesis studies suggest that the isoquinolinones and melatonin utilize different subsets of residues for receptor activation. A proposed binding cavity formed by a large group of hydrophobic amino acid side chains located at TM5, TM6 and TM7, may accommodate the aromatic substituent of isoquinolinone compound. This cavity includes the two conserved tyrosine residues on TM7 which appear to impose steric hindrance at the MT_1 receptor and result in a predominantly MT_2 selectivity profile for the isoquinolinones.

(1) Chan K et al (2013) Curr Med Chem 20: 289-300

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Intracellular Signalling Pathways In Mitochondrial Formylated Peptide-Induced Neutrophil Chemotaxis

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Introduction Mitochondrial formylated peptides (MTD) are released following necrosis and help drive neutrophil recruitment, via formyl peptide receptor 1 (FPR1), to sites of inflammation. Although FPR1-mediated intracellular signaling in response to bacterial formylated peptides is well understood different FPR1 ligands trigger distinct intracellular pathways.
Aim To determine the intracellular signalling pathways involved in MTD induced neutrophil chemotaxis.
Methods Human peripheral blood neutrophils were stimulated with isolated MTD (50µg/ml). MTD-induced neutrophil CD62L shedding and Mac-1 (CD11b/CD18) up-regulation and the effects of pharmacological inhibitors were assessed by flow cytometry. Inhibitors used were the FPR1 antagonist cyclosporin H (CsH; 5µM); the MAPK inhibitor PD0325901; the MEK inhibitor U0126; the p38 MAPK inhibitor SB203580; the PI3K inhibitors Wortmannin and LY294002; and the Akt inhibitor, Akti (all 10µM) or vehicle control. Neutrophil chemotaxis towards MTD was assessed over 90 minutes using a Boyden Chamber.
Results MTD induced CD62L shedding (control 1.00 vs MTD 0.2±0.1 ($n=3$, $p<0.05$)) and up-regulation of CD11b (control 1.00 vs MTD 2.0±0.2 ($n=3$, $p<0.05$)) and CD18 (control 1.0 vs MTD 2.3± 0.3 ($n=3$, $p<0.05$)) and These changes were inhibited by CsH. MAPK and PI3K inhibitors blocked changes in CD11b whilst Akti had no effect. CsH inhibited MTD-induced neutrophil chemotaxis (MTD 1055.0 ± 282.8 cells/ml vs MTD & CsH 183.5±89.3 ($n=3$, $p<0.05$)). In addition, an anti-CD11b blocking antibody inhibited neutrophil chemotaxis.
Discussion These data demonstrate that MTD induce neutrophil chemotaxis *in vitro* via the FPR1 receptor. Moreover, MAPK- and PI3K-signalling are involved in MTD-induced changes in neutrophil adhesion molecule expression, including expression of Mac-1, which is demonstrated to be critical for neutrophil chemotaxis. These findings provide a potential target for future therapeutics to treat sterile inflammatory conditions.

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The Effect of Ayurved Siriraj Wattana (AVS073) on COX-2 Expression by Lipopolysaccharide (LPS)-Treated Peripheral Blood Mononuclear Cells (PBMC).

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Introduction. Ayurved Siriraj Wattana (AVS073) is a Thai traditional medicine has been employed for health promotion and the prevention of age-related problems.
Aims. To investigate the effects of ethanolic extracts of AVS073 on COX expression by LPS-stimulated PBMC.
Methods. PBMC from whole blood were treated with LPS and measured cell proliferation using the MTT assay. COX-2 expression was measured after 2 to 24 hours induction. Anti-inflammatory effects of AVS073 were assessed by measuring the levels of COX-1, COX-2 mRNA and protein and PGE_2 production after LPS stimulation.
Results. AVS073 had no affect on cell proliferation and the optimum expression of COX-2 occurred at 18 hours after LPS. The highest concentration of extract was ound to significantly decrease COX-2 protein. Meanwhile, PGE_2 production appeared the significantly increased ($p<0.05$) when treated with AVS073 whereas COX activity did not change in PBMC.
Discussion. AVS073 was not cytotoxic at the concentration studied and appeared to possess anti-inflammatory effects at the pre-transcriptional level, indicated by down-regulation of COX-2 protein. A constituent of AVS073, *Terminalia chebula* (1), produced similar effects. However, the results of EIA assay exerted the possibility of PGE_2 production did not come from inhibited COX-2 protein, but may be produce from COX-1 protein.

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Interleukin-6 is Required for The Maintenance of Stemness in Lapatinib-Resistant Breast Cancer Cells

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Introduction. HER2-targeted therapies, such as the HER2 tyrosine kinase inhibitor (TKI) lapatinib (GW572016, Tykerb®) have been approved and shown significant clinical benefits for breast cancer patients. However, patients eventually developed acquired resistance, severely limiting the application of HER2-targeted therapy.
Aims. Understanding the mechanisms underlying acquired resistance is greatly helpful for developing novel strategies to circumvent the therapeutic hurdle.
Methods. Acquired lapatinib-resistant clones were cultured with DMEM/F12 containing 1μM lapatinib. Both mRNA and protein expression of IL-6 were examined by PCR and ELISA. The cell viability of these cells were examined by MTT. HER2 kinase activity, its major downstream signaling as well as IL-6 were detected via western blot. The cells were cultured in plates with ultra-low attachment and spheres were visualized and quantified under light microscopy. The Aldehyde dehydrogenase (ALDH) activity of these cells were examined by a colorimetric assay kit.
Results. The acquired lapatinib-resistant clones of HER2-positive cells continued to proliferate and maintain stemness properties even desipte loss of HER2 activity. IL-6 production was elevated in the resistant clones in comparison to their parental cells. Deprivation of IL-6 expression reduced the population of breast tumor initiating cells and subsequent cell viability of these lapatinib-resistant clones. Further analysis of downstream signaling driven by IL-6 revealed that STAT3 activation might be the major target. Interestingly, STAT3 activation also regulated IL-6 production, leading to the formation of an IL-6 inflammatory loop.
Discussion. The stemness control for cell survival is switched from HER2 to IL-6, conferring lapatinib resistance. Blockage of IL-6 may be a potential strategy to overcome lapatinib resistance.

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Treatment Of *Coscinium Fenestratum* Stem Extract In STZ-induced Diabetic Rat: Expression Proteomics Evaluation

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Introduction. Diabetes mellitus is a chronic metabolic disease defined by the level of hyperglycemia giving rise to risk of micro vascular damage. *Coscinium fenestratum* is a medicinal plant found in Southeast Asia (1). Earlier report on crude and partially purified fraction E from the DCM stem extract of *Coscinium fenestratum* showed plasma glucose reduction and antioxidant properties.
Aims. Current study was aimed at proteomics approach to study the mechanism of action of the extract.
Methods. 15 Sprague Dawley rats (250g) were injected with 35mg/kg of streptozocin (stz) to induce diabetes. After 1 week of observation, rats with plasma glucose level of 11mmol/L or 200mg/dL were orally administered with fraction E extract (100mg/kg) for 80 days, with blood glucose and weight measured weekly. After 80 days the rats were sacrificed and the pancreas was harvested, washed with distilled water and crushed to powder with liquid nitrogen and proteins were extracted. Two dimensional gel electrophoresis and PD Quest software genomic solutions investigator was used to analyze the differential protein expression profile in treated and untreated STZ-induced diabetic rat model. The proteins of interest were identified using Maldi-tof-tof-MS and bioinformatics protein software was used to identify the protein (1).
Results. The analysis showed substantial differential protein expression between the normal, negative, control and fraction E treated group. The proteins identified are mostly antioxidant proteins.
Discussion. The protein expression profile in Fraction E treated group was similar to the control group suggesting similar mechanism, which maybe linked to stimulation of antioxidants proteins. Further research is on going to identify the proteins.
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Endothelial Cell Senescence Attenuates Ca^{2+} Response And PGI_2 Production While Nitric Oxide Production Is Unaffected.

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Introduction. Endothelial dysfunction plays a crucial role in the pathogenesis of atherosclerosis which is a characteristic of vascular senescence.
Aims. We would reveal how cell senescence could affect endothelial Ca^{2+} response and the productions of nitric oxide (NO) and prostacyclin (PGI_2).
Methods. Subcultured (2nd-7th passage) porcine aortic endothelial cells (PAECs) were used as a cell senescence model. Bradykinin (BK, 10nM)-induced Ca^{2+} response was measured by fura-2/AM. BK-stimulated NO and PGI_2 production were measured by DAF-AM spectroscopy and 6-keto $PGF_{1\alpha}$ production, respectively. The responses to BK were compared between primary and subcultured PAECs.
Results. BK increased cytosolic Ca^{2+} concentration in primary PAECs. BK-induced Ca^{2+} response was attenuated in a cell passage-dependent manner (trend $p<0.01$). However, intracellular Ca^{2+} mobilization from endoplasmic reticulum was not influenced by cell senescence. BK increased the production of 6-keto $PGF_{1\alpha}$ in primary cultured PAECs. This production was significantly inhibited in the absence of extracellular Ca^{2+} and attenuated in cell senescence (trend $p<0.001$). BK increased the production of NO, which was inhibited in the absence of extracellular Ca^{2+}. However, BK-stimulated NO production was not influenced by cell senescence.
Discussion. Cell senescence attenuates Ca^{2+} response and PGI_2 production elicited by BK, which might be a causal mechanism leading to atherosclerosis.

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Dose-Related Effects of NMDA on Retinal Morphology and RGC Survival

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Introduction. Glutamate excitoxicity plays a major role in the loss of retinal ganglion cells (RGCs) in glaucoma. The toxic effects of glutamate on RGCs are mediated by the overstimulation of NMDA receptors. Intravitreal NMDA injections in varying doses are often used to study molecular mechanisms of ganglion cell death and neuroprotection. However, the dose-related effects of NMDA on retinal morphological changes remain unclear.
Aims. To evaluate the comparative dose-dependent effects of NMDA on retinal morphology and RGC survival using retinal morphometric measurements.
Methods. 150-200g Sprague Dawley rats were divided into 4 groups. Groups 1, 2, 3 and 4 were intravitreally administered with vehicle, NMDA 80 nmol, NMDA 200 nmol and 400 nmol, respectively. Seven days after NMDA injection, rats were sacrificed, eyes were enucleated, fixed and processed for H&E staining. The thickness, area and length of the inner retinal layer (IRL) and ganglion cell layer was measured and the number of cells counted. Subsequently, morphometric analyses were performed.
Results. Intravitreal NMDA injection particularly at higher doses caused severe degenerative changes with an initial stage of massive cellular swelling and, subsequently, RGC loss. GCL showed increasing reduction in thickness (µm) with 1.2, 2.0 and 2.2 fold reduction in groups 2, 3 and 4, respectively, compared to group 1. Although, volume density of RGCs in inner retina, did not show significant differences between four groups, significantly lower numeric density was observed in all NMDA treated groups compared to vehicle treated group.
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Changes in the minerals and trace elements concentration in the rat retina after NMDA exposure

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Introduction. Glutamate excitotoxicity is an important contributor to the death of retinal ganglion cells (RGCs). The toxic effects of glutamate on RGCs are mediated by the overstimulation of NMDA receptors due to their extreme permeability to calcium ions. Both decreased and elevated levels of minerals can cause retinal ganglion cell dysfunction. Among minerals, iron (Fe), zinc (Zn) and copper (Cu) are essential trace elements playing key roles in visual function. Zinc (Zn), copper (Cu) selenium (Se) are part of antioxidant defence mechanisms in retina.
Aims. To evaluate changes in the minerals and trace elements concentration in the rat retina after NMDA exposure.
Methods. 150-200g Sprague Dawley rats were divided into control group and group with intravitreally administered NMDA (80 nmol) (n=5). Seven days after NMDA injection, rats were sacrificed, eyes were enucleated, and retina was taken for minerals (Ca, Na, K, Mg) and trace elements (Mn, Cu, Fe, Se, Zn) content analysis using Inductively Coupled Plasma (DRC ICP-MS) techniques (NexION 300D).
Results. Intravitreal NMDA injection caused severe degenerative changes accompanied with increased mineral content such as Ca, Na and K by 3 times compared to control group. The trace elements also showed a significant increase amounting to 3-6 times compared to control group.
Conclusion: Intravitreal administration of NMDA results in significant changes in mineral and trace element contents of retina in rats.
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An *In Vitro* Study Of Compounds With Selectivity For Human Neuronal Sodium Channel Subtypes

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Introduction. Many neurological disorders involve abnormalities of neuronal excitability and abnormal function of voltage-gated sodium channels (Nav) have been implicated in some of these disorders (1). Axons contain Nav1.6 and Nav1.2 subtypes with different thresholds and functions and thus drugs selective for these subtypes could be interesting drug candidates for neurological disorders.
Aims. Novel compounds based on the known sodium channel blocker, mexiletine were synthesised and assessed for potency (IC50) by patch clamping Chinese Hamster Ovary cells expressing either recombinant human Nav1.2 or Nav1.6 ion channels and compared to the potency of mexiletine to identify compounds with subtype selectivity.
Methods. An automated patch clamp screening assay (2) in multiwell plates using an IonWorks Quattro instrument was used to measure the potency of compounds in 3 conformational states of sodium channels: (i) tonic closed state; (ii) frequency-dependent state (10 Hz) and (iii) slow inactivated state.
Results. Mexiletine was 2 to 3 times more potent in inhibiting Nav1.2 than Nav1.6 subtypes in the 3 conformational states. Interestingly one compound with a diphenyl substitution was 70-fold more potent on Nav 1.2 subtype than 1.6 subtype while another compound with a dipyridyl substitution was approximately 5 times more potent on Nav 1.6 than the 1.2 subtype and at least 20 to 30 fold more potent than mexiletine on both subtypes. Both compounds scored well as potential central nervous system active drugs by *in silico* calculations of 6 physico-chemical parameters (3) whereas mexiletine did not.
Discussion. Both compounds have *in vitro* pharmacological and pharmacokinetic properties that justify testing them as drug candidates in animal models of neurological disorders.

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Functional analysis of TNNI3K MAP kinase - From basic to clinical research

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We investigated roles of TNNI3K, a MAP kinase specifically and continuously expressed in cardiac muscles and interacted with cardiac troponin I, in the cardiac myogenesis process and in the repair of cardiac ischemic injury. TNNI3K high-expression enhanced cardiac performance, attenuated ischemia-induced ventricular remodeling in mice with myocardial infarction, and protects the myocardium from ischemic injury through suppressing phosphorylation of cTnI, annexin-V^{+}, Bax, and p38/JNK-mediated apoptosis. Intramyocardial administration of TNNI3K-overexpressing P19CL6 cells in mice with myocardial infarction (AMI) improved cardiac performance and attenuated ventricular remodeling. With a mutation of TNNI3K gene by substitution of serine at 835-836 sites with alanine, increased incidences of arrhythmias including tachycardia after are observed, indicating the elucidation of the TNNI3K-mutation mechanisms would be a new mechanism for cardiac-arrhythmias. In vitro experiments also indicated that TNNI3K-mutation increases incidences of early or delayed after-depolarization and prompts ryanodine-induced Ca^{2+}-oscillation through promoting ryanodine-induced Ca^{2+}-oscillations. Furthermore, availability of plasma TNNI3K level was investigated using with anti-TNNI3K antibodies in patients diagnosed as AMI, chronic heart failure and acute renal failure; and in healthy volunteers groups. Data shown that circulating TNNI3K levels were significantly higher in AMI when compared with other two groups($p<0.001$), indicating that measurement of circulating TNNI3K is a novel and useful diagnosing tool for AMI. In conclusion, using TNNI3K as a molecular target is a new potential approach for developing therapeutic or diagnostic agents for ischemic cardiac diseases by means of molecular biology techniques.

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Role Of Surface Residues On Defining The Functional Specificity Of Nm23 Metastasis Suppressors

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Introduction. Members of the human Nm23 family (also called NME) act as metastasis suppressors and they exhibit multiple enzymatic activities including phosphorylation of nucleoside diphosphates and DNA cleavage. Although highly similar, the two most extensively studied members, Nm23-H1 and Nm23-H2, display differential preferences for protein interactions (1). The resolved structures of Nm23-H1/2 revealed that several clusters of isoform-specific amino acids are located on exposed surfaces of these proteins.
Aims. To assess the role of surface clusters on Nm23-H1/2 as determinants of protein recognition.
Methods. Specific surface amino acids were swapped between Nm23-H1 and Nm23-H2 by site-directed mutagenesis. Wild-type and mutants were examined by co-immunoprecipitation (Co-IP) assays and functional characterizations in transfected cell lines.
Results. Nm23-H1 mutants with multiple mutations were generated: Q42R/H69N, Q42R/A62P/H69N, E124K/G131S, E124K/G131S/H135K, Q147H/N148D, T143K/Q147H/N148D, and L47H/E50Q. The mutants were successfully expressed in HEK293 and MDA-MB-231 cell lines. Protein partners of Nm23 showed differential binding preferences for wild-type Nm23-H1/2 and Nm23-H1 mutants.
Discussion. The successful expression of Nm23-H1 mutants in transfected cells suggests that the alteration of these outer surface residues did not significantly affect the overall structure of Nm23-H1. Preliminary data suggested that both Nm23-H1 and H2 could form protein complexes with Gβγ in Co-IP assay, but show different affinities for the A kinase anchor protein Lbc. Whether the surface clusters can play any role in determining the functions of Nm23 isoforms remains to be uncovered.

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Non-dissociable Gβ-Gα Fusion Proteins Reveal Different Mechanisms Of G protein Activation

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Introduction. Activation of heterotrimeric G proteins is commonly believed to trigger the dissociation of Gβγ dimers from GTP-bound Gα subunits. Such a model is mainly based on the observation of purified G proteins in non-physiological environments, with little supportive evidence in living cells. For several G proteins, unequivocal subunit dissociation upon activation could not be detected by FRET (1). Thus the traditional idea of complete subunit dissociation may need further validation or refinement.
Aims. To examine if subunit dissociation is universally required for the functional activation of G proteins.
Methods. Binding of different Gα subunits to Gβγ was assessed by co-immunoprecipitation assays using Flag-tagged $G\beta_1$ in the presence of GTPγS or GDPβS. Fusion proteins of Flag-tagged $G\beta_1$ linked to various Gα subunits (Gq, G11, Gs, Gi3, Gz) via a single amino acid or 16 residues were generated as previously described (2). Fusion proteins as well as their constitutively active (QL) mutants were examined for adenylyl cyclase or PLCβ activities in transfected cells.
Results. Upon activation by GTPγS, Gαq and Gαs remained bound to $G\beta_1$ whereas Gαi3 could not be co-immunoprecipitated from the same cell lysates. Fusion proteins with a single amino acid linking Gβ to constitutively active Gαq (β-1-αqQL) or Gα11 (β-1-α11QL) significantly stimulated PLCβ in COS-7 cells. Similarly, β-1-αsQL efficiently stimulated adenylyl cyclase. However, β-1-αi3QL and β-1-αzQL did not inhibit adenylyl cyclase but those with a long linker (β-16-αi3QL and β-16-αzQL) remained fully functional.
Discussion. The demonstrated functionality of the one-linker fusion proteins of Gαq, Gα11 and Gαs supports an activation mechanism without subunit dissociation. In contrast, fusion proteins of Gαi3 and Gαz suggest that these G proteins may require physical dissociation from Gβγ for effector regulation.

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Osteopontin Suppresses FcεRI Activation of Human Mast Cells by Inhibiting Chemokine, Th1 and Th2 Cytokines Releases

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Introduction. Osteopontin (OPN) is an RGD-containing extracellular matrix protein with post-translational modifications that can function in both soluble and matrix-fixed forms. This protein is upregulated in inflammatory diseases and important to their pathogenesis. However, OPN is also important in tissue repair and has protective roles during infections that make the functions of this protein debate for years.
Aims. Due to the importance of mast cells in inflammatory diseases, tissue repair and infection, we examined the effects of both soluble and matrix-fixed human milk OPN (hOPN) on human mast cells (HMC) stimulated by anti-IgE to simulate the allergic responses.
Methods. The adhesion, cytokines expression (respectively IL-8, TNF-α and IL-5), and histamine release of anti-IgE-stimulated human peripheral $CD34^+$ stem cells-cultured HMC were determined by CyQUANT cell proliferation assay, ELISA and spectro-fluorescence assay respectively.
Results. Matrix-fixed hOPN induced HMC adhesion and morphological change. Matrix-fixed hOPN also suppressed anti-IgE-induced IL-8, TNF-α and IL-5 release. In contrast, soluble hOPN did not affect anti-IgE-mediated responses. By employing blocking agents, we further demonstrated that the anti-IgE-mediated interaction of OPN and HMC was mediated by RGD domain of OPN and αVβ3 Integrin but not CD44 on HMC.
Discussion. These studies demonstrate that hOPN regulates adaptive immunity of HMC by suppressing anti-IgE-induced chemotaxis, th1 and th2 activation. (This project is supported by RGC Grant 469310.)

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The Effects Of Ayurved, Siriraj Ha-Rak (AVS022), And Its Components On COX Isoforms Expressed In IL-1β-induced HUVEC

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Introduction. AVS022 is composed of five herbal roots. It has long been widely used as an anti-inflammatory agent (1). However, its mechanism of action has not been previously reported.
Aims. To investigate the effect of AVS022, including its components, on the expression of COX1 and COX2 by IL-1β induced-HUVEC.
Methods. AVS022 and its components (1, 10 and 100 µg/ml) were incubated with IL-1β induced HUVEC for 24hr. Cell viability was tested using the MTT assay. COX-1, COX-2 mRNA and protein were assessed using real time PCR and Western blot, respectively. PGE_2 was measured to reflect COX activity using EIA.
Results. AVS022 had no effect on cell viability. AVS022 (1 and 10µg/ml) inhibited COX-1 mRNA and COX-2 protein. However, PGE_2 was increased from 8.7 ± 2.5 to 521.8 ± 11.3 pg/ml ($p<0.05$). Interestingly, individual components produced different effects.
Discussion. AVS022 and its components can modulate COX-2 at the pre-translational level. However, the increase PGE_2 may be preferable to COX-1 protein. The different effects of each component may suggest a biphasic dose-dependent action on COX activity.

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5,7-Dihydroxyflavone Augments Sorafenib-Mediated Cytotoxicity in Hepatocellular Carcinoma Cells

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Introduction. Sorafenib (Nexavar®, BAY43-9006) is currently the standard treatment for advanced hepatocellular carcinoma (HCC). However, the therapeutic efficacy is not optimal.
Aims. To develop alternative strategies for improveming sorafenib therapy in HCC.
Methods. The protein expression in HCC cells were examined by Western blot. Transfections of small-interfering RNA (siRNA) and DNA were conducted by using TransIT-2020 transfection reagent. According to the manufacturer's instructions, cells with 60-70% confluence were transfected with siRNA or DNA, followed by the indicated experiments. The cell viability assays were conducted by crystal violet staining (1).
Results. Chrysin increased the level of ERK1/2 phosphorylation in both time- and dose-dependent manners. Furthermore, molecular analysis revealed that the chrysin-synergized anti-growth effect by sorafenib in HCC cells was enhanced by overexpression of MEK, a positive regulator of ERK1/2 phosphorylation. However, this synergistic effect was lost when MEK activity was blocked by the U0126 inhibitor. In parallel, chrysin could also enhance sorafenib-mediated cytotoxicity when the ERK1/2 phosphorylation was increased by silence of dual-specificity phosphatase 2 (DUSP2), which is a negative regulator of ERK1/2 phosphorylation.
Discussion. Sustained ERK1/2 activation by chrysin renders HCC cells more sensitive to sorafenib treatment. Chrysin may be a potential sensitizer to elevate the anti-tumor activity of sorafenib in HCC cells in the future.

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The IUPHAR/BPS Guide To PHARMACOLOGY (GtoPdb): An Expert-Driven Knowledgebase Of Drug Targets And Their Ligands

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Introduction. The IUPHAR/BPS Guide to PHARMACOLOGY (GtoPdb; http://www.guidetopharmacology.org) is an open access database providing pharmacological, chemical, genetic, functional and pathophysiological data on the targets of drugs. It includes literature-derived, quantitative data on the actions of approved and experimental compounds at their targets.
Aims. GtoPdb aims to provide concise overviews of the key properties of a wide range of established and potential drug targets, with nomenclature information, selective ligands and background reading.
Methods. Developed under the auspices of the International Union of Basic and Clinical Pharmacology (IUPHAR) and the British Pharmacological Society (BPS), the data are curated by an international network of >650 expert contributors coordinated by the IUPHAR Nomenclature Committee (NC-IUPHAR).
Results. GtoPdb includes data on >2700 targets and their interactions with ligands. Targets are divided into families and the information is summarised on a single page. More detailed information and drug lists are provided for a subset of important targets. The data are linked to other databases, including Entrez, UniProt, ChEMBL, DrugBank, PubChem and reference citations in PubMed. Each of the 7200 small molecule and peptide ligands is manually annotated with 2D chemical structures or amino acid sequences, nomenclature and clinical information for approved drugs. 'The Concise Guide to PHARMACOLOGY' (http://www.guidetopharmacology.org/concise) is a biennial publication 'snapshot' of the database with the key properties of each target family, intended as a quick desktop reference guide.
Discussion. Current work includes completing the annotation of all the targets of currently approved drugs, as well as those in development and the likely future candidate targets.

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Targeting STAT3 Signaling Pathway In Hepatocellular Carcinoma: Recent Findings With Natural Agents Emodin And Garcinol

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Introduction: Signal transducers and activators of transcription (STATs) comprise a family of cytoplasmic transcription factors that transmit signals, mediate intracellular signaling that is usually generated at cell surface receptors and then transmitted to the nucleus. Numerous studies have demonstrated constitutive activation of STAT3 in a wide variety of human tumors, including hematological malignancies (leukemias, lymphomas, and multiple myeloma) as well as solid tumors (such as head and neck, breast, lung, gastric, hepatocellular, colorectal and prostate cancers). There is strong evidence to suggest that aberrant STAT3 signaling promotes development and progression of human cancers by either inhibiting apoptosis or inducing cell proliferation, angiogenesis, invasion, and metastasis. However, the development of novel drugs for the targeting STAT3 that are both safe and efficacious remains an important scientific and clinical challenge.
Aims: To test the potential STAT3 modulatory effects of emodin and garcinol in hepatocellular carcinoma.
Methods: The effect of emodin and garcinol on STAT3 activation, associated protein kinases, and apoptosis was investigated using various HCC cell lines. Additionally, the *in vivo* effect of these drugs on the orthotopic/xenograft mouse models was also examined.
Results: We will present recent data from our group that shows that novel small molecule inhibitors (garcinol/butein) can suppress STAT3 activation through diverse molecular mechanisms and modulate the expression of genes involved in cancer progression.
Discussion: Our findings have identified two novel STAT3 inhibitors with promising anticancer effects.

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Use of an NLuc-BRET System to Investigate Ligand-Binding to the Human β_1-adrenoceptor

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Introduction. Bioluminescence resonance energy transfer (BRET) is a tool for measuring protein-protein and ligand-receptor interactions (1). In this report we have determined whether a 19kDa luciferase NanoLuc (NLuc; Promega) from the deep sea shrimp *Oplophorus gracilirostris* can be expressed on the N-terminus of the human β_1-adrenoceptor (β_1-AR) and used to monitor ligand-receptor binding with a fluorescent ligand.
Aims. To use propranolol-PEG8-BY630 (Prop-BY630;(2)) to investigate ligand-receptor interactions at the human β_1-AR.
Methods. Experiments were conducted in 96-well plates containing HEK 293 cells transfected with the human NLuc-β_1-AR. Cells were grown to confluence, growth media was removed and then replaced with 100µl HBSS. Prop-BY630 (100nM) was added in the presence or absence of competing ligands and incubated at 37°C for one hour. The substrate furimazine was then added to the plate and after 5 minutes read on a BMG PheraStar (filters: 460-80nm, 610nm-LP).

Table 1 - Competition binding with 100nM Prop-BY630

Compound	-log K_i (mean ± SEM)	n	-log K_i[3,4]
ICI 118551	-6.53 ± 0.19	4	-6.5
Cimaterol	-6.55 ± 0.11	7	-6.6
Isoprenaline	-6.71 ± 0.16	7	-6.1
Propranolol	-8.30 ± 0.11	4	-8.2
CGP 20712a	-8.55 ± 0.04	7	-8.8
CGP 12177	-8.70 ± 0.05	7	-9.2

Results. Specific binding of Prop-BY630 (log K_D = -7.06 ± 0.06, n=8) was observed to the human NLuc-β_1-AR. Both β-AR agonists and antagonists were able to compete with this binding (Table 1).
Discussion. These data demonstrate the ability of BRET-based systems to quantify ligand-receptor interactions at the human β_1-AR pharmacology in living cells.

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Hepatoprotective Effects Of Ultrasonication Processed Ginseng Berry Extract On D-GalN/LPS-induced Acute Liver Failure Model in Rats

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Introduction. Acute hepatic failure (ALF) is life-threatening critical condition in which rapid deterioration of liver function, and liver transplantation is known to only effective therapy for ALF. Several studies have shown that *Panax Ginseng* have hepatoprotective effects. In this experiment, ultrasonication processed ginseng berry extract (UBE) was used.
Aims. Evaluates the hepatoprotective effect of UBE in a rat acute liver failure model.
Methods. Male Sprague-Dawley rats weighing 220-230 g divided into 6 groups (9 rats per group). Group 1 and 2 were administered normal saline (10 ml/kg, p.o.), Group 3 rats were administered with silymarin (150 mg/kg, p.o.) and Group 4, 5, 6 rats were administered with UBE (100, 250, 500 mg/kg, p.o.) for 4 weeks. Rats were sacrificed 24 h after D-galactosamine/lipopolysaccharide (D-GalN/LPS; 300 mg/kg / 30 μg/kg, i.p.) challenge or normal saline administration (group 1; 1 ml/kg, i.p.). Animal experiments were approved by the Institutional Animal Care and use Committee of Chung-Ang University (CAUIACUC-14-0031).
Results. Pretreatment with UBE significantly decreased ALT, AST and bilirubin levels in D-GalN/LPS challenged rats in a dose-dependent manner. In addition, TNF-α and heme oxygenase−1 levels in liver were also significantly decreased in UBE pretreated group. Antioxidative enzymes such as superoxide dismutase, glutathione peroxidase and catalase activities were significantly increased by UBE treatment. Liver histopathology also showed that UBE treatment reduced the centrilobular necrosis and inflammatory cell infiltration evoked by D-GalN/LPS challenge.
Discussion. UBE had hepatoprotective effect on ALF model in rat. UBE may have great potential to be developed as a new functional food by prevention of ALF.

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The Design, Synthesis, And Antitumor Activity Evaluation Of A Novel Combi-chloroethylnitrosourea Prodrug Simultaneously Releasing A DNA Crosslinking Agent And A O^6-alkylguanine-DNA alkyltransferase Inhibitor

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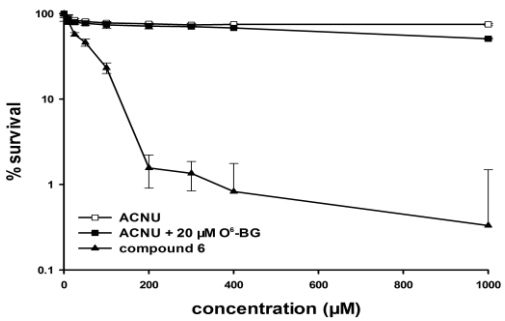
Introduction. The resistance mediated by O^6-alkylguanine-DNA alkyltransferase (AGT) in tumor cells limits the efficiency of chloroethylitrosourea (CENUs) which exert their activity through forming dG-dC interstrand crosslinks (ICLs) (1). Therefore, the suppression of AGT in tumor cells is a prerequisite for efficient chemotherapy by CENUs.
Aims. The synthesis of a novel combi-chloroethylnitrosourea prodrug 3-(3-(((2-amino-9H-purin-6-yl)oxy)methyl)benzyl)-1-(2-chloroethyl)-1-nitrosourea 6 simultaneously releasing a DNA crosslinking agent and an AGT inhibitor, and evaluating its antitumor efficiency.



Methods. Three glioma cells, SF126, SF767, and SF763, were selected for antitumor activity evaluation by CCK8 assay. The clinically widely used CENUs, ACNU, BCNU, and their combination chemotherapy with O^6-benzylguanine (O^6-BG) were conducted as positive control.
Results. The novel synthesized combi-chloroethylnitrosourea 6 obtained lower IC_{50} than ACNU, BCNU and the combination chemotherapy.
Discussion. The superior antitumor activity of novel synthesized combi-chloroethylnitrosourea 6 may be due to the dual release of a DNA crosslinking agent chloroethyldiazonium ions and an AGT inhibitor O^6-BG analogue which leads to enhanced dG-dC crosslinking levels.

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Anti-inflammatory Effects of Compound A from *Atractylodes Lancea* (Thunb.) DC. in Dengue Virus Infection

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Introduction. Dengue virus is one of the most important mosquito-borne human pathogen that causes a serious public health problem to the people who live in tropical and subtropical regions of the world including Thailand. A great deal of evidence suggests that an increased risk of severe disease is mediated by immunopathological mechanisms which create excessive immune activation. Currently, neither a vaccine to prevent nor an effective therapeutic agent to treat dengue infection is available.
Aims. To investigate antiviral and anti-inflammatory activities of compound A (CpdA) from *Atractylodes lancea* (Thunb.) DC. against dengue virus infection.
Methods. Antiviral and anti-inflammatory activities against dengue virus infection of CpdA were evaluated by determining its effect on cellular viral load, viral replication, viral production, and cytokine expression and secretion profile after the cells were infected with dengue virus.
Results. Result suggests that CpdA from *A. lancea* (Thunb.) DC. rhizome significantly reduced dengue virus induced cytokine expression and secretion.
Discussion. CpdA is a promising candidate for using as anti-inflammation in combination with anti-dengue virus agents against dengue virus infection.

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CCL5 promotes vascular endothelial growth factor-dependent angiogenesis in human osteosarcoma

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Introduction. Chemokine CCL5 has been reported to facilitate tumor progression and metastasis. Previous studies also showed that CCL5 directly promotes angiogenesis of endothelial cells and chemotaxis of human endothelial progenitor cells (EPCs). However, the crosstalk between CCL5 and vascular endothelial growth factor (VEGF) as well as tumor angiogenesis in human osteosarcoma microenvironment has not been well explored.
Aims. We investigated the relationship of CCL5 with VEGF expression and tumor angiogenesis, and further elucidated its mechanism of action in human osteosarcoma.
Methods. We designed the basic and translational studies to investigate the role of CCL5 on VEGF-mediated angiogenesis in the cell experiments, animal models and clinical patients.
Results. We found that CCL5 increased VEGF expression in human osteosarcoma cells and subsequently induced migration and tube formation in human EPCs. CCL5 promoted VEGF expression and angiogenesis through CCR5, PKCδ, c-Src, and HIF-1α signaling cascades. In addition, knockdown of CCL5 suppressed VEGF expression and abolished osteosarcoma conditional medium-induced angiogenesis *in vitro* and *in vivo*. CCL5 knockdown significantly inhibited tumor growth and angiogenesis in osteosarcoma microenvironment using tumor xenograft model. Importantly, we demonstrated that the expression of CCL5 and VEGF were correlated with tumor stage according the immunohistochemistry analysis of human osteosarcoma tissues.
Discussion. Our findings provide evidence that CCL5-CCR5 axis promotes VEGF-dependent angiogenesis in human osteosarcoma microenvironment through PKCδ/c-Src/HIF-1α signaling pathway. This is the first indication that CCL5 induces tumor angiogenesis by VEGF production in human cancer cells.

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Interaction Between IP, TP, and 5-HT$_2$ Receptors During Contractions to Prostacyclin in Rat Renal Arteries

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Introduction. Endothelium-dependent contractions are augmented in isolated arteries of hypertensive animals. Prostacyclin has been identified as a major endothelium-derived contracting factor (EDCF) in preparations of the spontaneously hypertensive rat (SHR) – activating TP receptors on vascular smooth muscle cells, but the role of IP receptors is unknown.
Aims. To define the role of IP receptors in renal arteries in EDCF-mediated responses.
Methods. Isometric tension was recorded in Halpern-Mulvany myographs in quiescent (L-NAME 300 µmol/L) renal artery rings of SHR and normotensive Wistar Kyoto (WKY) rats (n=6).
Results. SHR renal artery rings contracted significantly more upon exposure to 60 mmol/L high potassium (high K^+) depolarizing solution compared to WKY (16.6±0.6 vs 13.8±0.5 mN; P<0.001). The augmented EDCF-mediated responses to acetylcholine in SHR (74±5% vs 44±3% of high K^+ in WKY) were abolished by 10 µmol/L indomethacin and 100 nmol/L S18886. They were not affected by the IP receptor antagonist CAY10441 (1 µmol/L) in SHR but significantly increased in WKY (65±3% high K^+; P<0.01) preparations. This compound also facilitated contractions to exogenous prostacyclin (in the presence of indomethacin, P<0.001) in arteries from both strains and unmasked contractions to the prostanoid during TP receptor blockade with S18886 (abolishing U46619-induced contractions also in the presence of CAY10441). These fully preserved contractions to prostacyclin during combined IP and TP receptor blockade were prevented by the 5-HT$_2$ receptor antagonist ketanserin (1 µmol/L).
Discussion. These findings suggest that the presence of IP receptors critically affects EDCF-mediated responses, and that contractions of renal arteries partially depend on 5-HT$_2$ receptor activation besides TP receptor signalling, when IP receptors are inhibited. Amplifying effects of endogenous serotonin may be involved in the regulation of renal artery vasomotor tone by prostanoids.

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Oxidative Stress in Metabolic Diseases: Role of Antioxidant Nutraceuticals As Adjuvant Therapy

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Introduction. Visceral adiposity is considered an important source of oxidative stress (OS), which may play a key role in CVD development (1), carcinogenesis and angiogenesis. In the obese, adipose tissue is highly infiltrated with macrophages and, circulating PBMC are activated (2), contributing to increased OS as a result of high levels of ROS and reduced antioxidant status. Selenium (Se), an essential micronutrient, is incorporated in antioxidant Glutathione Peroxidase (GPx) enzymes which protect cells from OS and cell damage.
Aims. To investigate the effect of Se supplementation in modulating OS state representative of obese individuals and regulating angiogenic processes.
Methods. U937 monocyte cells were supplemented or not with Se and OS was induced by addition of 1mM paraquat (PQ)/0.7mM SNAP. Cell viability, ROS generation and GPx1-4 gene expression were assessed. A co-culture system of primary human endothelial cells and fibroblasts was used to study angiogenesis and MCAM antibody staining to assess tubule formation.
Results. PQ/SNAP treatment significantly reduced U937 cell viability and increased ROS generation compared to untreated control. Supplementation with 100nM Na_2SeO_3 significantly increased cell viability by 33% and significantly reduced ROS generation by 32% in cells treated with PQ/SNAP. Correspondingly, GPx1-4 genes expression was increased by 146% and 77%, respectively. Se showed 95% inhibitory effect on tubule formation after 7 days incubation compared to untreated co-culture.
Discussion. Se supplementation may be effective in counteracting OS by significantly increasing antioxidant genes expression: enhancing, therefore, endogenous antioxidant protection to quench ROS generation more effectively and improve cell viability. Data from this *in vitro* study provides support for using Se as nutraceutical and adjuvant therapy to minimize OS damaging effects in metabolic diseases.

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Brain Oxidative Stress in Neural Mechanism of Programmed Hypertension to Maternal High Fructose Diet

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Introduction. Early life exposure to adverse environments can lead to a variety of adult diseases by a process referred to as developmental programming. Maternal high fructose diet (HFD) promotes programmed hypertension in adult offspring, although the underlying mechanism is not fully understood. Brain oxidative stress is pivotal in neural mechanism of hypertension.
Aims. We tested the hypothesis that brain oxidative stress contributes to programmed hypertension in adult offspring to maternal HFD.
Methods. Female normotensive Sprague-Dawley rats fed with HFD during gestation and lactation periods were used. Metabolic parameters were measured by biochemical assay in the offspring at the age of 3 months old. Blood pressure was monitored under conscious condition by the tail-cuff method. Tissue superoxide level was measured by electron spin resonance (ESR) spectroscopy. Test agents were given orally to mother during lactation or to the offspring at the age of 2-month-old.
Results. Maternal HFD during gestation and lactation led to insulin resistance, increase in sympathetic activity and hypertension in the 3-month-old young offspring. This was associated with augmented neurogenic sympathetic vasomotor tone and high tissue levels of superoxide at the brain stem. Maternal melatonin treatment or simvastatin treatment to the 2-month-old offspring significantly ameliorated oxidative stress in the brain stem, abrogated sympathetic overexcitation and prevented the development of programmed hypertension in the 3-month-old rats to maternal HFD.
Discussion. Maternal HFD induces an early onset of high blood pressure, which is associated with oxidative stress in the brain stem. Both maternal treatment with melatonin or simvastatin to the young offspring ameliorate brain oxidative stress and promote antihypertension. These results suggest that brain stem oxidative stress may contribute to programmed hypertension in the offspring to maternal HFD.

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Wy14643 and Fenofibrate Reduce Contractions to Hydrogen Peroxide in Aortae of Spontaneously Hypertensive Rats

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Introduction. Oxidative stress, with the production of hydrogen peroxide, contributes to the development of vascular dysfunction in hypertension (1). Hydrogen peroxide evokes contractions in many blood vessels, including rat aortae and renal arteries, and rabbit pulmonary arteries (1). Recent studies showed that Wy14643 and fenofibrate, agonists of peroxisome proliferator-activated receptor alpha, improve endothelial function in vascular diseases (2).
Aim. The present study was designed to examine whether or not Wy14643 and fenofibrate inhibit hydrogen peroxide-induced contractions in aortae of spontaneously hypertensive rats (SHR).
Method. Male SHR and their normotensive counterparts, Wistar Kyoto rats (WKY), of 40-44 weeks old were used. Thoracic aortic rings, with and without endothelium, were isolated and suspended in organ chambers for isometric tension recording.
Results. Hydrogen peroxide evoked endothelium-independent contractions in both SHR and WKY; the contraction was significantly greater in rings of SHR than in those of WKY, suggesting that hydrogen peroxide plays a role in vascular dysfunction in hypertension. Wy14643 and fenofibrate significantly reduced hydrogen peroxide-induced contractions in SHR rings with, but not in those without, endothelium, indicating that the inhibitory effect was endothelium-dependent.
Discussion. The smooth muscle of aortic rings of SHR has a higher sensitivity to hydrogen peroxide-induced contractions than WKY. Wy14643 and fenofibrate act on the endothelium to reduce hydrogen peroxide-induced contractions in hypertensive rat aortae.

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Exogenous Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) Pathway Increases Atherogenesis

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Introduction. Deletion of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) gene increased atherosclerosis in mice, indicating a vascular protection role of TRAIL. The effect of exogenous TRAIL on early atherogenesis is unknown.
Aims. In the present study, we examined the effects of systemic TRAIL administration on early atherogenesis in vivo.
Methods. We delivered recombinant TRAIL into apolipoprotein E (ApoE)-deficient and LDL receptor-deficient mice via i.p. injection once a week.
Results. We found that exogenous TRAIL had a stimulating effect on atherogenesis. This effect was unrelated to changes in plasma lipids, the type of diet used, or the phenotype of circulating monocytes. Blocking inflammatory chemokine signalling with neutralizing antibodies against CCL2 and CCL5 abolished the proatherogenic effect of TRAIL. Using DR5 receptor/ApoE double knockout mice, we showed that the proatherogenic effect of TRAIL was DR5-dependent.
Discussion. We provided first evidence showing that activation of the TRAIL/DR5 pathway promoted atherogenesis in hyperlipidemic mice. Given the potential usefulness of TRAIL in clinical therapies, our results raise the possibility that TRAIL treatment may be associated with an increased risk of atherosclerosis.

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Low Dose Combination Antihypertensive Pharmacotherapy Can Be Effective. Angiotensin Receptor Blockers / Angiotensin Converting Enzyme Inhibitors Often Unnecessary

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Introduction. Hypertension treatment often fails to achieve goal blood pressure (BP), despite a wide range of available pharmacotherapy. Poor compliance can be due to side effects, which are often dose-related. Combination therapy with antihypertensive drugs at half-standard dose has been shown to reduce side effects and increase efficacy, so-called 'polypill'. Although angiotensin inhibitors (angiotensin receptor blockers and angiotensin converting enzyme inhibitors) are popular, significant side effects include increased BP variability which may predispose to falls and probably accounts for their inferior efficacy against stroke, compared to diuretics and calcium channel blockers (CCBs) which reduce BP variability.
Aims. To assess which antihypertensive pharmacotherapy can control BP in practice.
Methods. We examined medical records of 50 consecutive patients in a cardiovascular clinic, selected if the current supine systolic BP was < 145 mm Hg (measured by an automatic device) and there was a history of hypertension controlled for at least 6 months with stable pharmacotherapy and no side effects. Participants had previously tried a range of antihypertensive agents.
Results. Mean age was 72 years, mean current BP 135/78 mm Hg, women:men 29:21, more than half had symptomatic artery disease (42% coronary disease, 12% previous stroke, 12% claudication), 30% had renal impairment (eGFR < 60). Mean weight was 78 kg and 36% were diabetic. Systolic BP was a mean of 32 mm Hg lower than the highest known BP (167 mm Hg), on a median of 3 (range 1-6) antihypertensive drugs, mostly at lowest recommended doses. 80% of participants were on diuretic, 78% on CCBs, 50% on beta-blocker and only 38% on an angiotensin inhibitor.
Discussion. Despite advanced age, arterial and renal disease, low dose antihypertensive pharmacotherapy can control moderately severe hypertension. Diuretics and calcium channel blockers were the most commonly utilised antihypertensive drug classes and appeared well tolerated.

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Investigation of Directly and Indirectly Mediated Cardiovascular Actions of Stimulants

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The stimulants cathine and cathinone (from Khat leaves) and methylhexanamine (MeHex) produce adrenoceptor mediated tachycardia and vasopressor actions that are probably the result of direct receptor stimulation and displacement of noradrenaline from nerve terminals. Given the widespread use of these agents, and the resultant adverse effects, we wished to establish the importance of indirect actions in the overall cardiovascular effects of these agents in vivo.
Male Wistar rat were anaesthetized with pentobarbitone (60mg/kg, i.p.). Studies were approved by the HPRA and by the RCSI Research Ethics Committee. Some rats were sympathectomised with 6-hydroxydopamine (40mg/kg i.p., day 1 & 4, used on day 5 or 6). Dose-response curves were obtained to test drugs given i.v.. Data is presented as mean±S.E.M., (n animals), and analysis was performed using ANOVA and Dunnett's test.
In anaesthetised rats, cathine, cathinone, MeHex and tyramine (all 0.001-1mg/kg) produced marked tachycardia, and cathine, MeHex and tyramine produced marked pressor responses. In sympathectomised rats, the tachycardia to all agents was markedly attenuated. Blood pressure effects of cathine, MeHex and tyramine were also markedly attenuated by sympathectomy.
The results demonstrate that chemical sympathectomy greatly reduces responses to all agents investigated, demonstrating that all act as indirect sympathomimetics.

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Deletion of SIRT1 in perivascular adipose tissue accelerates obesity-induced endothelial dysfunction

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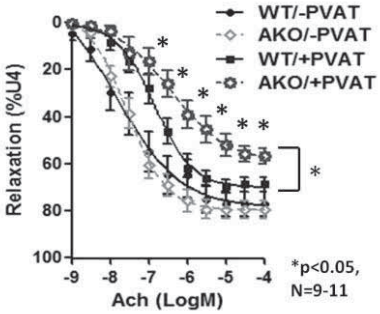

Introduction. Perivascular adipose tissue (PVAT) exhibits brown adipose features and its dysfunction is implicated in cardiovascular diseases. SIRT1 is key to adipocyte phenotypes and metabolism.
Aims. In the present study, we aim to investigate the role of SIRT1 within PVAT in modulating obesity-evoked endothelial dysfunction.
Methods. Wild type (WT) and adipocyte-specific SIRT1 knockout mice (AKO) were fed with standard chow or westernized diet for 12 weeks. Endothelium-dependent relaxation (EDR) in aortic rings with or without PVAT was assessed by wire myograph. DHE staining was used to measure superoxide.
Results. In the presence of PVAT, the EDR was significantly impaired in aorta from obese mice, and such an impairment was further exacerbated in obese AKO mice. In accompany, AKO mice exhibited exacerbated atherosclerosis in apoE-/- background. PVAT in lean WT mice displayed a brown phenotype, whereas SIRT1 deficiency augmented obesity-induced brown-to-white transition. In WT mice, chronic cold exposure (4ºC for 1 week) reversed obesity-induced attenuation of brown phenotypes, thereby leading to improved vascular reactivity by reducing superoxide and increasing adiponectin. However, all these beneficial effects of chronic cold exposure were abrogated in AKO mice..
Discussion. The brown phenotype of PVAT is associated with increased endothelial functions. SIRT1 plays a pivotal role in controlling PVAT browning, which in turn causes decreased superoxide production and increased adiponectin to protect vascular injury.

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Effects of Isoflavones on the Release of Inflammatory Mediators by Cigarette Smoke in Airway Epithelial Cells

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Introduction. Chronic obstructive pulmonary disease (COPD) is characterized by airway inflammation, and is associated with cigarette smoking. The morbidity and mortality of COPD patients remain high, despite the currently available pharmacological treatments.
Aims. The present study investigated the potential of isoflavones in reducing airway inflammation.
Methods. Inflammation was induced in human bronchial epithelial BEAS-2B cells by exposure to cigarette smoke medium (CSM). These cells were incubated with or without different isoflavones [daidzein, genistein, genistin, glycetin and puerarin], the anti-inflammatory glucocorticoid, dexamethasone, or the extracellular signal-regulated kinase (ERK) inhibitor, U0126. The amounts of the inflammatory mediators, interleukin (IL)-8 and monocyte-chemotactic protein-1 (MCP-1), in the culture medium was measured with enzyme immunoassays.
Results. CSM (4%) stimulated the release of IL-8 and MCP-1 by BEAS-2B cells after 24 hours. Dexamethasone (1 μmol/L) and U0126 (10 μmol/L) inhibited the cigarette smoke-induced release of these inflammatory mediators. Among the five isoflavones tested, only genistein, at 3 and 10 μmol/L, inhibited the release of IL-8 from BEAS-2B cells. Genistein (10 μmol/L) also reduced the MCP-1 release from the cells.
Discussion. The findings, therefore, suggest that genistein has an anti-inflammatory effect on airway epithelial cells. In view of the involvement of ERK in cigarette smoke-induced inflammation, it is possible that genistein may cause inhibition of this enzyme to reduce airway inflammation.

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Investigation of the Antiarrhythmic Mechanism of the Multi-herbal Medicine Xing Su Ning

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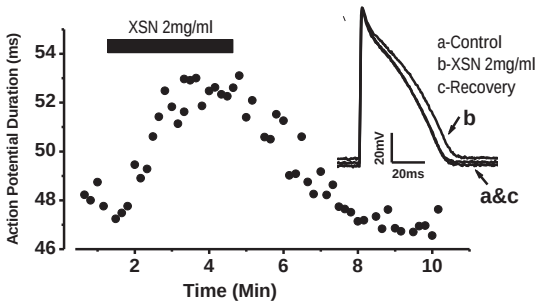
Introduction. Xing Su Ning (XSN) is a multi-herbal Chinese medicine produced by Momentum Pharmaceutical Co. Ltd., which is sold in China since 2005 for treating cardiac ventricular arrhythmia, especially arrhythmias induced by cardiac ischemia and viral myocarditis.
Aims. To discover the cellular electrophysiological mechanism of the actions of XSN in treating cardiac arrhythmia.
Methods. Whole-cell patch-clamp techniques (1) were used to record action potentials and whole cell current in isolated adult rat ventricular myocytes. The myocytes were continuously perfused with physiological solution without or with XSN.
Results. XSN at 2mg/ml significantly prolonged the action potential Duration (APD) as shown in the Figure: control was 45.4ms±4.9 and with XSN 2mg/ml was 52.2ms±4.5 (P<0.01, n=7). At this concentration XSN did not have significant effect on the resting potential or the amplitude of the action potential. The effect of XSN is reversible upon the washout of the medicine.



Discussion. XSN Prolongs APD, an action that increases the effective refractory period which suppress tachyarrhythmias caused by reentry mechanisms. XSN displays the property of Class III antiarrhythmic drugs, such as amiodarone, without adverse reactions. Further studies on the effect of XSN at various concentrations on action potential and ionic channels, i.e. potassium channels, will be carried out.

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Effects of Taurine on Contractions of Human Internal Mammary Artery

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Introduction. Taurine is a sulphur containing amino acid derived from cysteine. It is widely distributed in human and animal tissues. It is suggested that taurine has important roles in regulation of vascular tone. Studies in isolated tissue baths show that taurine relaxes precontracted arteries.
Aims. Human internal mammary artery (IMA) is the graft of choice for coronary artery by-pass surgery (CABG) because of its high patency rates compared to other grafts. This study aimed to show the effects of taurine on IMA in vitro and explain the it's mechanism(s) of action.
Methods. The response in the IMA was recorded isometricaly by a force displacement transducer in isolated organ baths. Taurin (20, 40 and 80 mM) was added to organ baths after precontraction with KCl (45 mM) or serotonin (5-HT, 30 μM). Taurine-induced relaxations were tested in the presence of the large conductance Ca^{2+}-activated K^+ channel inhibitor tetraethylammonium (TEA, 1 mM), ATP-sensitive K^+ channel inhibitor glibenclamide (GLI, 10 μM), the voltage-sensitive K^+ channel inhibitor 4-aminopyridine (4-AP, 1 mM) and inward rectifier K^+ channel inhibitor barium chloride ($BaCl_2$, 30 μM). All experiments were also performed in solutions containing the cyclooxygenase inhibitor indomethacin (10 μM) and the nitric oxide synthase inhibitor L-NAME (100 μM).
Results. Taurine did not affect the resting tone of IMA. However, it produced relaxation in 5-HT and KCl precontracted preparations. The relaxation to IMA was not affected by the K^+ channel inhibitors GLI, TEA, $BaCl_2$, and indomethacin or L-NAME. 4-AP significantly inhibited taurine-induced relaxations ($p<0.05$).
Discussion. The preincubation of IMA with taurine antagonized KCl and 5-HT induced contractions in a concentration-dependent manner, while it did not affect the resting tone. The relaxations to taurine were almost abolished by pretreatment with 4-AP. These results show that the mechanism of action of taurine may be via activation of voltage-sensitive K^+ channels.

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Lipocalin-2 Deficiency Attenuates Atherosclerosis Development by Preventing Neutrophil Activation and Vascular Inflammation

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Introduction. Atherosclerosis is a progressive inflammatory disorder of the arterial wall, characterized by endothelial damage, adhesion and migration of monocytes, lipid accumulation in macrophages and formation of foam cells. Lipocalin-2 is a pro-inflammatory protein causatively involved in insulin resistance, endothelial dysfunction and hypertension caused by obesity.
Aims. The present aimed to determine whether or not lipocalin-2 contributes to the development of atherosclerosis.
Methods. Male apolipoprotein E-deficient mice with (ApoE-/-) or without (ApoE-/-Lcn2-/-) lipocalin-2 gene expression were fed high fat high cholesterol (HFHC) diet for 30 weeks. Metabolic parameters were measured biweekly and atherosclerotic plaque formation determined at the end of treatment.
Results. The results revealed that lipocalin-2 deficiency significantly attenuated the deterioration in circulating lipid profile and the development of atherosclerotic lesions in ApoE-/- mice. Indeed, neutrophils derived from lipocalin-2 deficient mice did not form neutrophil extracellular trap (NET) upon stimulation with linoleic acid. Exogenous lipocalin-2 administered together with linoleic acid activated neutrophils derived from ApoE-/-Lcn2-/- mice and enhanced NET formation. These results suggest that beneficial effects of lipocalin-2 deficiency in preventing vascular inflammation can be attributed to reduction in neutrophil activation.
Discussion. Lipocalin-2 contributes to the development of atherosclerosis by promoting neutrophil activation and chronic vascular inflammation.

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Exendin-4 Attenuates Experimental Diabetic Cardiomyopathy via Direct Actions on Inflammation

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Introduction. Increasing evidence indicates that the insulinotropic peptide hormone, glucagon-like peptide-1 (GLP-1), also exerts important cardiovascular actions. However, its role in the diabetic heart is unclear.
Aims. To study effects of the GLP-1 mimetic, exendin-4 (Ex-4), on experimental diabetic cardiomyopathy.
Methods. Streptozotocin (STZ)-induced diabetic and control mice (n≥7) were chronically infused with or without Ex-4 (25nmol/kg/day) prior to detailed *in vivo* / *ex vivo* analysis and complementary cell studies.
Results. At 12 weeks, Ex-4 reduced blood HbA1c (STZ 12.4±0.7% vs STZ Ex-4 9.4±0.7%, P<0.01) and triglycerides in diabetic mice, and conferred protective effects on pancreatic islets. These effects were associated with improved echocardiographic diastolic function in STZ Ex-4 treated mice (mitral valve E/A: STZ 1.2±0.1 vs STZ Ex-4 1.5±0.1, P<0.001), which importantly, was not evident in an insulin comparator group with equivalent metabolic changes. Improved cardiac function in Ex-4 treated mice was associated with preferential reduction of interstitial fibrosis (STZ 6.0±0.4% vs STZ Ex-4 4.4±0.4%, P<0.05) and modulation of extracellular matrix gene expression (e.g. MMP-2), together with specific attenuation of early (4 weeks) myocardial macrophage infiltration (flow cytometry: STZ 20.6±1.4% vs 15.5±1.1%, P<0.05). Interestingly, *in vitro* studies demonstrated that whilst Ex-4 (1nmol/L) had no effect on TGF-β induced differentiation of murine cardiac fibroblasts, Ex-4 directly modulated basal inflammatory gene expression in murine bone marrow derived macrophages (e.g. IL-10: Con 1.0±0.1 vs Ex-4 2.0±0.3, P<0.01) under both normal (5.5mmol/L) and high glucose (25mmol/L) conditions. Furthermore, cardiac fibroblast differentiation in the presence of high, but not normal glucose, was inhibited by conditioned media from Ex-4 treated macrophages in which the expression of several key proteins was differentially modulated (e.g. MIP-1α, CXCL10).
Discussion. Ex-4 reduces experimental diabetic cardiomyopathy via preferential extracellular matrix effects, which appear to occur secondary to direct modulation of inflammation. These findings highlight specific targeting of GLP-1 signalling as a potential novel therapeutic strategy for heart failure in diabetes.

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Pharmacological inhibition of protein tyrosine phosphatase 1B promotes endothelial cells on cell spreading and migration

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Introduction. Several studies have suggested that protein tyrosine phosphatase 1B (PTP1B) regulates cell mobility. Moreover, PTP1B has also been implicated in regulating angiogenesis. However, its role in modulating endothelial cell spreading and migration is unclear.
Aims. We studied the effects of a specific PTP1B inhibitor C26H19Br2N3O7S3 in vascular endothelial cells in culture.
Methods. Human microvascular endothelial cells (HMVEC) were treated with PTP1B inhibitor C26H19Br2N3O7S3 at 10 μM.
Results. Endogenous expression of PTP1B in HMVEC was detected by western blot. Treatment with C26H19Br2N3O7S3 increased HMVEC cell spreading, formation of lamellipodia, and cell migration, whereas cell proliferation was not changed. PTP1B inhibitor had no significant effects on the tyrosine phosphorylation level of Src, FAK or paxillin, but significantly increased the activity of Rac1. Treatment of cells with the Rac1 inhibitor C24H38Cl3N7, but not the Rho inhibitor Rhosin, suppressed the effects of PTP1B inhibitor on cell spreading and migration. Although inhibition of VEGFR2 with VEGFR2 inhibitor suppressed the basal level of cell spreading, PTP1B inhibitor still increased the cell spreading response in the presence of VEGFR2 inhibitor.
Discussion. Our results suggest that inhibition of endogenous PTP1B promoted ECs migration and spreading likely through Rac1 activation. Our results raised the possibility that PTP1B inhibitor may be a useful drug to promote angiogenesis in clinical therapies.

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Endothelial SIRT1 Prevents Pathological Vascular Remodeling By Enhancing The Degradation Of Acetylated LKB1 Via HERC2

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Introduction. Vascular remodeling during aging is characterized by thickening and reduced compliance of the arterial wall. Liver Kinase B (LKB1) is a pro-senescence protein kinase which plays an important role in regulating vascular structure and functions. The anti-ageing protein Sirtuin-1 (SIRT1) elicits its beneficial effects in part by promoting proteasomal degradation of LKB1.
Aims. To determine the molecular mechanisms underlying SIRT1-regulated LKB1 degradation focusing on an E3 ligase, HERC2 (HECT domain and RLD 2 protein).
Methods. Two LKB1 mutants were created by mutation at lysine 64 to glutamine (K64Q) or arginine (K64R) and were transfected in primary cultures of porcine aortic endothelial cells (PAECs). The conditioned media from transfected PAECs was used to culture porcine coronary artery smooth muscle cells (PCASMC). Pressure myography was used to measure changes in isometric tension in rings of carotid arteries of eNOS-/- mice.
Results. LKB1 K64Q enhanced, whereas K64R attenuated the ubiquitination and nuclear degradation of LKB1. In PAECs, K64Q caused a reduction of cellular growth by promoting apoptosis, whereas K64R prevented it by inducing cellular senescence. K64Q promoted the proliferation of PCASMC. Abnormal arterial remodeling demonstrated by reduced wall thickness, stiffness and collagen accumulation was accompanied by an increased LKB1 expression and was attenuated by endothelial overexpression of SIRT1. These beneficial effects of SIRT1 were prevented by lentivirus-mediated knockdown of HERC2, which elevated vascular expression of acetylated LKB1 levels.
Discussion. SIRT1 prevents pathological vascular remodeling by promoting HERC2-mediated ubiquitination and degradation of acetylated LKB1.

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Distribution Of Lipocalin-2 In The Pericardium Of Cardiovascular Disease Patients

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Introduction. The proinflammatory protein lipocalin-2 is an injury marker for ischemic and chronic heart diseases. Wild type human lipocalin-2 (hLcn2) is modified by polyamines. A mutant with cysteine 87 residue replaced by alanine (C87A) exhibits reduced polyamination. Two polyclonal antibodies, anti-hLcn2 and anti-C87A, were generated to recognize wild type human lipocalin-2 and C87A lipocalin-2, respectively.
Aims. To evaluated the expression and distribution of lipocalin-2 in pericardial fluid and plasma and pericardium from patients with cardiovascular diseases.
Methods. Samples were obtained from 37 patients undergoing coronary artery bypass graft and/or valve replacement surgery. ELISA and immunohistochemical staining were used to test the lipocalin-2 level.
Results. Lipocalin 2 level detected by anti-C87A were almost one fold higher in fluid and four times higher in plasma than those detected by anti-hLcn2.The pericardial space appeared to be a microenvironment in which the lipocalin-2 level cannot be predicted by its circulating level. Proteins recognized by anti-hLcn2 were mainly present in blood cells and endothelium, while those detected by anti-C87A were present in the mesothelium, perivascular adipocytes, pericardial adipocytes, vascular smooth muscle cells and endothelium. Blood cells with positive lipocalin-2 staining tended to be attached to the vascular wall.
Discussion. The different distribution of lipocalin-2 variants may relate to the progress and/or status of cardiovascular disease.

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Dental Resin Curing Blue Light Induced Contraction Of Rat Aorta: Role Of Hydrogen Peroxide

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Introduction. Dental resin curing blue light (BL, a visible light) has been used for tooth bleaching and to restore teeth with resin-based composite fillings. UV light has been reported to induce photorelaxation via nitric oxide (1). Recent studies have shown that BL might induce oxidative stress with reactive oxygen species production (2). However, little is known about its vascular actions.

Aims. To investigate whether BL affected vascular reactivity in isolated rat aorta with and without endothelium and the mechanism of its effect.

Methods. Rat aorta rings were (n=12) irradiated for 5 min at a distance of 10.0 mm with a BL source (400-520 nm) in organ baths containing Krebs-Henseleit solutionwere recorded with isometric transducer and polygraph. In first set of experiments, responses to acetylcholine (ACh, 1 nM-1 μM) after precontraction with phenylephrine (PE, 1 μM) were obtained.and compared in BL-irradiated and control rat aorta rings (n=6). In a second set of experiments, effect of BL irradiation on vascular tone of aorta rings after PE precontraction reached to plateau was also evaluated and compared to control rings (n=6). Effect of incubation with catalase (1200 u/ml) on the responses to ACh and PE in BL-irradiated and control rings were also evaluated in adjacent aorta rings. In another set of experiments, total oxidative stress (TOS) and total antioxidant capacity(TAC) in BL-irradiated (n=6) and control(n=6) rat aorta preparations were measured with assay kits and spectrophotometry.

Results. BL did not significanty alter basal tone in endothelium intact or - denuded rat aorta rings. BL significantly decreased ACh - induced and endotelium - dependent relaxations in PE (1 μM) - precontracted rat aorta rings(n=6, $P<0.05$). Besides, BL induced additional contraction in isolated rat aorta ring segments precontracted with PE (1 μM). The contractile effect of BL was inhibited by 1200 u/ml catalase (n=6, $P<0.05$). We also found that BL irradiation increased the level of TOS in rat aorta rings (n=6, $P<0.05$) TAC did not differ between BL - irradiated and control preparations

Discussion.These results suggest that BL induced contraction of rat aorta may be mediated by elevated hydrogen peroxide (H_2O_2) production. We have previously shown that resin - based dental materials may induce vasodilation which may lead bleeding during dental treatments (3). Clinically, this opposing vasoconstictor effect of dental resin curing BL may be useful. Oxidant H_2O_2 may also impair endothelial functions as previously shown (4).

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Robust Heterologous Cross-Tolerance between Systemic Ethanol and Intracerebellar (ICB) Δ^9-THC, adenosine A_1-, $GABA_A$-receptor agonists and nicotine, using mouse cerebellar ataxia as a test response.

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Introduction. Alcohol and marijuana, often used in combination, are the most widely used psychoactive substances worldwide. Both are commonly detected in victims of traffic accident. Virtually no study has examined the effect of co-administered systemic ethanol and intracerebellar (ICB) Δ^9-THC on cerebellar ataxia (CA). Therefore, study of the combined effects of Δ^9-THC and ethanol on CA is of particular concern. Aims. (i) evaluate effect of repeated ICB Δ^9-THC/CHA/muscimol/nicotine on acute ethanol-induced CA and *vice versa;* (ii) formulate possible common underlying molecular mechanism(s) in CA. Methods**.** Ataxia (Rotorod) was used as a test response in male CD-1 mice. All drugs (Δ^9-THC 20 μg; adenosine A_1 agonist, CHA 8 ng; $GABA_A$ agonist, muscimol 20 ng; nicotine 5 ng) were infused directly into the cerebellum via guide cannulas, except ethanol (2g/kg; ip). Chronic drug treatment involved once daily x 5 days. Acute treatment involved a single administration 16 h after last chronic treatment. Results. Chronic Δ^9-THC infusions virtually abolished acute ethanol-induced CA and its potentiation and attenuation by CHA, muscimol, and nicotine, respectively, indicating robust heterologous cross-tolerance between THC and ethanol/adenosine A_1-, $GABA_A$-, and nACh-receptor signaling. Similar cross-tolerance was observed when the order of drug treatment was reversed (i.e., chronic ethanol instead of Δ^9-THC treatment), followed by acute CHA/muscimol/Δ^9-THC/nicotine. Cross-tolerance was diminished following twice vs. once daily chronic Δ^9-THC dosing, indicating some CB_1 receptor desensitization. Discussion. Repeated activation of $G_{i/o}$-coupled CB_1 and A_1AR receptors by chronic Δ^9-THC or CHA treatment, respectively, is known to upregulate cAMP during withdrawal. Chronic ethanol during withdrawal likely to upregulate nNOS and glutamate, with probable GABA downregulation. Similarly, GABA down regulation is likely following chronic muscimol treatment during withdrawal. Finally, chronic nicotine increases NO production and glutamatergic transmission. Therefore, the primary common molecular event in all these drug treatments appears to be disruption of central homeostasis between GABA and glutamate neurotransmission consistent with literature.

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Neuroprotective And Neurite Outgrowth-promoting Activities By Bis(propyl)-cognitin Via The Activation of Alpha7 Nicotinic Acetylcholine Receptor

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Introduction: The cause of Alzheimer's disease (AD) could be ascribed to the progressive loss of functional neurons in the brain, and hence agents with neuroprotection and neurite growth-promoting activities for compensating for neuronal loss may have significant therapeutic value.
Aims: The neuroprotective and neurite outgrowth-promoting activities and molecular mechanisms of bis(propyl)-cognitin (B3C), a multifunctional anti-AD dimer, were investigated.
Methods: MTT assay was used to assess cell viability, immunocytochemical staining was applied to evaluate the pro-neuritogenesis effects, western blot and short hairpin RNA assays were performed to explore the underlying mechanisms.
Results: B3C (IC_{50}, 0.08 μM) fully protected against glutamate-induced neuronal death in primary cerebellar granule neurons. The neuroprotection of B3C could be abrogated by methyllycaconitine, a specific antagonist of alpha7-nicotinic acetylcholine receptor (α7-nAChR). In addition, B3C significantly promoted neurite outgrowth in both PC12 cells and primary cortical neurons, as evidenced by the increase in the percentage of cells with extended neurites as well as the up-regulation of neuronal markers growth-associated protein-43 and β-III-tubulin. Furthermore, B3C rapidly upregulated the phosphorylation of extracellular signal-regulated kinase (ERK), a critical signaling molecule in neurite outgrowth that is downstream of the α7-nAChR signal pathway. Specific inhibitors of ERK and α7-nAChR, but not those of p38 mitogen-activated protein kinase and c-Jun NH(2)-terminal kinase, blocked the neurite outgrowth as well as ERK activation in PC12 cells induced by B3C. Most importantly, genetic depletion of α7-nAChR significantly abolished B3C-induced neurite outgrowth in PC12 cells.
Discussion: B3C provided neuroprotection and neurite outgrowth-promoting activities through the activation of α7-nAChR, which offers a novel insight into the potential application of B3C in AD treatment.

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Novel 5-HT_3 Receptor Antagonist (4-Phenylpiperazin-1-Yl) (Quinoxalin-2-Yl) Methanone (4a) Reverses The Altered Plasma Corticosterone, Leptin And Brain Oxidative Stress In Depression Co-Morbid With Obesity In Mice

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Introduction. The biological mechanisms that link the association of depression to obesity still remains a puzzle (1,2). The antidepressant-like effect of potential 5-HT_3 receptor antagonists is well evident from several pre-clinical studies (3).
Aims. To investigate the effect of (4-phenylpiperazin-1-yl) (quinoxalin-2-yl) methanone (4a), a novel 5-HT_3 receptor antagonist on depression co-morbid with obesity by performing behavioral and biochemical assays in mice.
Methods. Male Swiss albino mice fed with HFD for 14 weeks were administered with 4a (2 and 4 mg/kg, p.o.)/escitalopram (ESC) (10 mg/kg, p.o.)/vehicle (10 ml/kg, p.o.) for 28 days. Forced swim test (FST), plasma corticosterone (CORT), leptin, brain malondialdehyde (MDA) and reduced glutathione (GSH) were measured.
Results. Chronic treatment with 4a reversed the behavioral and biochemical alterations in obese mice.
Discussion. In summary, 4a exhibited antidepressant-like effect on depression co-morbid with obesity. The probable mechanism of action suggests that, 4a acts by allosteric modulation of serotonergic system.
Conclusion. 4a plays an important role in management of depression co-morbid with obesity in obese mice by reversing the altered HPA axis activity, leptin concentration and brain oxidant/antioxidant balance in brain.

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Neuroprotective Action Of GSCF and Dizocilpine in Cerebral Ischemia Induced Neuronal Injury

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Introduction: Acute cerebral ischemia remains a second leading cause of death and disability and vexing health problem worldwide. Previous evidences state that glutamate excitotoxicity was the early impact and main trigger that will root the tissue damage after cerebral ischemia. Halting or delaying the neurodegeneration after cerebral ischemia might have the greatest clinical impact and became a challenge to the neuroscientist.
Aim: The aim of the present study was to investigate the neuroprotective effects of combination of dizocilpine an NMDA receptor antagonist with a neurotrophic factor, G-CSF on ischemia/reperfusion (I/R) induced cerebral stroke.
Methods: We have induced the cerebral ischemia in mice using bilateral common carotid artery occlusion. The morphological (brain infarct size, cerebral edema) and astrocytic and microglial activation was evaluated in study groups.
Results: GCSF and dizocilpine have shown a significant attenuation of cerebral ischemia induced increase in infarct size and cerebral edema. Immunoreactivity of CD11b (a marker of astrocytic activation) was attenuated by G-CSF as well as G-CSF plus dizocilpine treatment and mild reaction was observed in dizocilpine alone treated animals. Both GCSF and dizocilpine reduced the GFAP expression in brains of mice.
Discussion: In ischemic stroke, resident brain macrophages/ microglia are activated within minutes after the injury involving the primary infarct area as well as remote sites. GFAP expression is a characteristic of reactive astrogliosis which are abruptly increased after CNS trauma, ischemic stroke and neurodegenerative diseases due to destruction of nearby neurons. We observed considerable increase in Immunoreactivity of microglia and astrocytes in infarct area after cerebral ischemia. Our observation of a decrease in GFAP Immunoreactivity in infarct area after treatment is consistent with other reports associating improved stroke recovery with reduced astrocytic reactivity.

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Tianeptine Differentially Affects Oxidative Phosphorylation and Neuroprotection Compared with Other Antidepressants.

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Introduction. We have shown that BDNF produced a concentration-dependent increase in respiratory control index (RCI, a measure of respiratory coupling efficiency, ATP synthesis) in mouse forebrain mitochondria co-incubated with synaptosomes, and the effect correlated with neuroprotection. The effects was mediated by TrkB-MEK-Bcl-2-complex 1 and were susceptible to inflammatory cytokines (1). Furthermore stress or chronic administration of corticosterone blocked RCI in frontal cortex of rats (2).
Aims. We assess if stress and antidepressants may modify RCI and be neuroprotective.
Methods. Metabolism of mouse forebrain mitochondria, with or without synaptosomes were incubated with glutamate/malate (complex1) or succinate (complex II).
Results. Sertraline, S-citalopram, imipramine, venlafaxine, fluoxetine (10μM) did not affect RCI on complex 1 or 2, whereas tianeptine was inactive on complex1 but increased RCI from 5.7 to 7.5. This was a direct effect on mitochondria and the signalling cascades of synaptosomes were not needed (as opposed to BDNF). Increasing concentrations of the other antidepressants either had no effect or collapsed mitochondrial membrane potential. In new-born mice, chronic administration of IL-1beta (10 ng/kg i.p. for 5 days) prior to ibotenate injection exacerbated lesion size in cortex and white matter, and this exacerbation was prevented by acute administration of tianeptine (10 mg/kg i.p.) concomitantly with ibotenate (3) but the other antidepressants were inactive or lethal in this model of coadministration with IL1beta at 10 mg/mg i.p.. No protective effect was seen without IL1beta exacerbation.
Discussion. These results show clearly that tianeptine has neuroprotective effects, over and above those of other antidepressants, probably mediated via mitochondrial efficiency (RCI).

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Depolarisation-induced gene changes in isolated dorsal root ganglion cells

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Introduction. Lipopolysaccharide (LPS) acts in a Toll-like receptor-4 (TLR4)-dependent manner to increase COX-2, IL-1β and TNFα mRNA expression in dorsal root ganglion (DRG) cells (1). PGE_2 also increases COX-2 and IL-1β, but inhibits TNFα mRNA expression (1). Intriguingly, LPS was also reported to depolarise DRG neurones.
Aims. We therefore wanted to determine if other depolarising stimuli produce similar changes in inflammatory gene expression as observed with LPS in DRG cells.
Methods. Rat DRG cells (mixed cultures of neurones and glial cells) were incubated in duplicate for 2 h with 50 mM KCl or 1 μg/ml ultrapure LPS in F14 culture medium containing 4% Ultroser G, then mRNA was extracted and assayed by real time PCR, using β-actin as the reference housekeeping gene.
Results. Both KCl and LPS increased COX-2 mRNA by 5.0-fold and 9.1-fold, respectively. However, while LPS increased both IL-1β and TNFα mRNA expression (10.2 and 13.1-fold, respectively), KCl inhibited IL-1β (0.4-fold) and had little effect on TNFα mRNA expression (1.3-fold).
Discussion. The profile of inflammatory gene expression in response to a classical depolarising stimulus (KCl) is different from the profile of responses observed for either LPS or PGE_2. The neuronal expression of COX-2 and its rapid induction by seizures or NMDA-dependent synaptic activity has been known for some time (2), therefore ongoing studies are also testing the inflammatory gene expression in DRG cells in response to glutamate. [This work was supported by a CUHK Direct Grant (4054159).]

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No Evidence of a Role for Renal Eicosanoid-Producing Cytochrome P450 Enzymes in Cadmium-Linked Kidney Disease

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Introduction. Cadmium (Cd) is an environmental pollutant which is associated with significant kidney dysfunction in exposed populations. This is due to the preferential accumulation of this metal in the kidney proximal tubules where, over-time, it causes toxicity. Animal studies have clearly shown a link between Cd exposure and hypertension where it is proposed that Cd induces renal eicosanoid-producing cytochrome P450 (CYP) expression which results in higher levels of vaso-constrictive molecules.
Aims. To determine Cd toxicity and its effect on expression of CYP (4A11, 4F2, 3A4, 2B6, and 2E1), metallothionein (MT), and heme oxygenase-1 (HO-1) in primary cultures of human proximal tubular cells (PTC) compared with the hepatoblastoma cell line HepG2 and the proximal tubular cell line HK-2.
Methods. PTC, HK-2, and HepG2 were cultured with different concentrations of Cd over 48 h. Cell toxicity was determined using the WST-1 cytotoxicity assay, lactate dehydrogenase release, and [3H]-thymidine incorporation. Expression of the five CYP and HO-1, as well as MT was determined by immunoblotting. Expression of the CYPs was also assessed in human kidney tissue (n=6) using immunohistochemistry.
Results. Only CYP4F2, CYP3A4 and CYP2B6 were detected in cultured cells. Cd concentrations above 10 μM caused significant cell death in the three cell types. Exposure of the cells to Cd (5 μM) for 48 h had no effect on the levels of CYP4F2, CYP3A4 and CYP2B6. Expression of MT and HO-1 was, however, induced by Cd in a concentration-dependent manner. All tissue sections expressed CYP4A11, CYP4F2, CYP3A4, CYP2B6, and CYP2E1 with strong staining in proximal tubule cells.
Discussion. The *in vitro* study does not support a role for these CYPs in Cd-induced hypertension.

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The Effect Of *Garcinia mangostana* Linn. Extract On Modulation of *Plasmodium falciparum* Prostaglandin

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Introduction. Development of new promising antimalarial drugs is urgently needed due to the emergence of resistance of malaria parasite to almost all of the available drugs.
Aims. The aim of the present study was to investigate the effect of *Garcinia mangostana* Linn. (pericarp) crude extract on the production of prostaglandin in 3D7 clone *P. falciparum*.
Methods. The parasite clone was exposed to *Garcinia mangostana* Linn. (pericarp) crude extract for 12 hours and levels of the three prostaglandins, i.e., PGE_2, PGD_2, and $PGF_{2\alpha}$ produced were determined using HPLC.
Results. The levels of all prostaglandins were significantly decreased in parasite exposed to the extract compared with control.
Discussion. The crude extract of the *Garcinia mangostana* Linn. possesses several pharmacological activities including anti-inflammatory activity. The inhibitory activity on prostaglandin production in cancer cells has been demonstrated (1,2). The current study demonstrated its inhibitory activity on prostaglandin production in malaria parasite.

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Influence of *CYP3A* Polymorphisms on the Pharmacokinetics of Oral Midazolam and the Urinary 6β-Hydroxycortisol/Cortisol Ratio as Markers of CYP3A Activity in Healthy Male Chinese

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Introduction. The oral plasma clearance of midazolam and the ratio of 6β-hydroxycortisol (6β-OHF) to cortisol (F) in urine are two potential markers for evaluating CYP3A activity *in vivo*.
Aims. We assessed the influence of two common *CYP3A* polymorphisms on the pharmacokinetics of oral midazolam and urinary ratio of 6β-OHF/F in healthy Chinese.
Methods. Single oral 15-mg doses of midazolam were given to 20 healthy male Chinese subjects who were genotyped for the *CYP3A5* *3 and *CYP3A4* *1G polymorphisms. Morning urine samples were collected after overnight fasting. The plasma concentrations of midazolam were determined by LC/MS/MS and urine F and 6β-OHF concentrations were measured using UPLC.
Results. There were no significant correlations between the pharmacokinetic parameters of midazolam and urinary ratio of 6β-OHF/F. The *CYP3A* polymorphisms examined had no significant associations with the urinary ratio of 6β-OHF/F or the pharmacokinetics of midazolam. However, diplotype analysis showed that CYP3A5 expressers with the *CYP3A4* *1/*1G genotype (n = 3) had significantly lower midazolam $AUC_{0-\infty}$ values (210.0 ± 33.5 vs. 313.9 ± 204.6 h·ng/mL, $P < 0.05$) and higher CL/F values (1.16 ± 0.16 vs. 0.88 ± 0.48 L/h/kg, $P < 0.01$) compared to subjects with the *CYP3A4* *1/*1 genotype (n = 4).
Discussion. There were no significant associations between midazolam pharmacokinetic parameters and urinary ratio of 6β-OHF/F and the two *CYP3A* polymorphisms had no effect on the urinary ratio of 6β-OHF/F or midazolam pharmacokinetics. However, *CYP3A4* *1G polymorphism might increase the activity of CYP3A among CYP3A5 expressers, but not for CYP3A5 non-expressers and that the clearance of midazolam is likely to be influenced by the combined effects of *CYP3A4* and *CYP3A5* polymorphisms, which is consistent with some previous studies with tacrolimus. The potential interaction of *CYP3A5* *3 and *CYP3A4* *1G polymorphism requires confirmation in a larger study.

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Anti-oxidative Efficacy of Crude Water Extract of Mangosteen Peel (CWM) in Alzheimer's Patients

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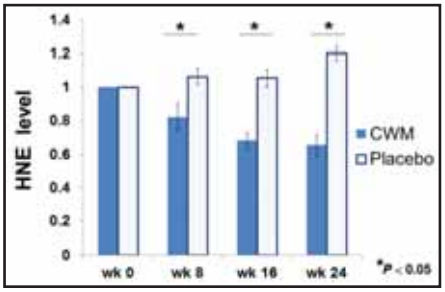

Introduction. Oxidative stress has been reported to be involved in Alzheimer's disease (AD). 4-Hydroxy-2-nonenal (HNE), a lipid peroxidation product, was reported to be a biomarker in this disease (1). Theoretically, the therapeutic use of free radical scavengers (or antioxidants) may be useful in treating AD (2).
Aims. To evaluate the anti-oxidative efficacy of 24 weeks administration of a crude water extract of CWM in AD patients compared with those who received placebo.
Methods. Two groups of subjects were given CWM (n=13) or placebo (n=9) orally once daily: 280 mg (weight > 60 kg) and 220 mg (weight < 60 mg). Blood samples were collected at week 0, 8, 16 and 24, and HNE levels determined by dot blot evaluation. Mann-Whiteney U test was used for statistical analysis. A *P*-value less than 0.05 was considered to be statistically significant.
Results. CWM administration significantly reduced oxidative status compared to placebo in AD patients from week 8 onwards.
Discussion. The phenolic content of CWM possesses anti-oxidative properties in AD patients. Therefore, this extract has the potential to be developed for clinical use (in combination to conventional medicines) in the treatment of AD.

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Effects Of General Practitioner Education On The Prescription Of Anticholinergic Medications

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Introduction. Increasing general practitioner (GP) awareness of the additive anticholinergic effects of various drugs (anticholinergic load) and of the potential of these drugs to act as a reversible cause of cognitive impairment, may reduce anticholinergic load in the elderly.
Aims. To examine the effects of a GP-based educational intervention on the anticholinergic load of Australian community dwelling elderly patients over a 2-year period.
Methods. Participants aged 75 years or older (n=272), were recruited at four sites (Newcastle, Sydney, Melbourne, and Adelaide). A research nurse visited the home of each patient to compile a list of current medications, and assess their cognitive status using a subsection of the revised Cambridge Examination for Mental Disorders of the Elderly (CAMCOG-R; 1). Anticholinergic load was determined using the Anticholinergic Drug Scale (2). Intervention GPs received education on diagnosis and management of dementia including identification of reversible causes of cognitive impairment (3).
Results. The control group showed a significant increase in anticholinergic load over 2 years (0.8 ± 0.14 vs 1.18±0.20; n=61, P=0.028) and a significant decrease in CAMCOG (86.5±1.01 vs 83.0±1.72; n=61, P=0.013). The intervention group showed no significant difference in anticholinergic load over 2 years (1.2±0.1 vs 1.1±0.1; n=211) and the CAMCOG showed a small but significant decline (86.9±0.5 vs 85.8±0.8; n=211, P=0.036).
Discussion. This dementia-focused GP education prevented an increase in anticholinergic load. A larger study using a more targeted approach involving pharmacists and practice nurses as well as GPs may achieve a decrease in anticholinergic prescribing.

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The protein kinase inhibitors trametinib and dabrafenib inhibit human UDP-glucuronosyltransferase 1A4 and 1A9

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Introduction. Trametinib and dabrafenib are recently developed protein kinase inhibitors that are used for the treatment of metastatic melanoma with a BRAF V600 mutation. Although trametinib and dabrafenib have been investigated as inhibitors of cytochrome P450 enzymes and membrane transporters, the effect of these drugs on human UDP-glucuronosyltransferase (UGT) activities is unknown.
Aims. This study aimed to characterise the inhibitory effects of trametinib and dabrafenib on human UGT enzymes and to assess the drug-drug interaction potential.
Methods. The inhibitory potency of trametinib and dabrafenib was determined using recombinant human UGT (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17) enzymes. Concentrations of trametinib and dabrafenib were corrected for nonspecific binding to the enzyme source (HEK293 cell lysate) in kinetic studies that determined K_i values ($f_{u,HEK}$ values were approximately 0.8).
Results. Trametinib and dabrafenib inhibited UGT 1A1, 1A4, 1A7, 1A9 and 1A10 activities by >50% at inhibitor concentrations of <20 µmol/L. In subsequent kinetic studies the most potent inhibition was observed with UGT1A4 and 1A9; trametinib inhibited lamotrigine N_2-glucuronidation by rUGT1A4 and propofol glucuronidation by rUGT1A9 with respective K_i values of 0.2 and 6.1 µmol/L, while dabrafenib inhibited propofol glucuronidation by rUGT1A9 with a K_i value of 0.3 µmol/L.
Conclusion. Trametinib and dabrafenib are potent inhibitors of UGT1A4 and 1A9, enzymes that contribute to the glucuronidation of numerous drugs and non-drug xenobiotics.

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Polypharmacy, age-dependent physiological changes and risk of falls in older people

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Introduction. Falls in people aged 65 years and over have been recognised as a major public health issue because of their morbidity, mortality and cost to the healthcare system. Psychotropic medications have been identified as the leading cause of falls in older people; however, there is evidence to suggest that an increase in incidence of falls is associated with other medication classes.
Aims. This study aims to investigate the link between exogenous (medication classes) and endogenous (age-dependent physiological changes) factors that can lead to falls in older people (≥65 years of age).
Methods. Using a retrospective clinical audit design, the medical records of 250 randomly selected older patients admitted to a public teaching hospital following a fall were examined. Age, gender, patients' regular medications, renal and hepatic function, and neurological function were documented. Data were analysed using IBM SPSS version 21. Chi-square analysis was deployed to examine associations between explanatory variables and the risk of falls. The study was approved by the Human Research Ethics Committee of Melbourne Health.
Results. Of the 238 patients who met the inclusion criteria, 96% were on regular medications. The most commonly used medication classes were analgesics, lipid-modifying agents, psychotropic and anti-hypertensive medications. There was a significant association between patients' impaired renal function and anti-hypertensive medication use χ^2 (138) = 172, p=0.026. Furthermore, there were significant associations between cognition and number of regular medications used, χ^2 (34) = 76, p=0.018, and patients' age and hepatic function χ^2 (1140) = 1298, p=0.001.
Discussion. In this study anti-hypertensive medication use, cognitive decline and changes in hepatic and renal function have been identified as risk factors associated with falls. The association with anti-hypertensive medications was due to age-related changes which resulted in diminished ability to excrete drug metabolites. Cognitive deficits were directly related to the number of regular medications used.

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Protective effects of CHA79, a synthetic chalcone derivative, on methylglyoxal-induced neurotoxicity

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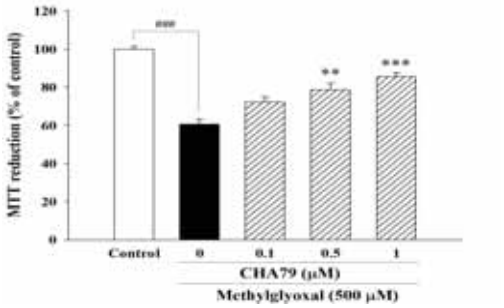

Introduction. Methylglyoxal (MG), the most potent precursor of advanced glycation end products, plays a key role in the pathogenesis of neurodegenerative diseases. CHA79, a synthetic chalcone derivative with iodo substitution at position 2 on A-ring, possesses inhibitory effect on cellular glucose consumption.
Aims. This study investigated the effects of CHA79 on MG-induced toxicity in neuronal SH-SY5Y cells.
Methods. SH-ST5Y cells were treated with CHA79 for 1 h, and then added MG for 24 h. Cell viability was measured by MTT assay. Apoptotic death was evaluated by Hoechst33342 staining and observed under inverted microscope. Protein expression was detected by western blots analysis.
Results. MG concentration-dependently attenuated cell viability of SH-ST5Y cells. However, CHA79 significantly increased cell viability of MG-treated SH-ST5Y cells. CHA79 attenuated MG-induced apoptotic death via modulating expression of anti-apoptotic and apoptotic proteins. Moreover, CHA79 upregulated expressions of insulin-like growth factor-1 receptor, glucagon-like peptide-1 receptor, p-Akt and p-GSK-3β in MG-treated SH-SY5Y cells. Furthermore, CHA79 activated p75 neurotrophin receptor (p75NTR)/BDNF signal pathway.
Discussion. CHA79 attenuated MG-induced neurotoxicity, providing a new direction for drug development on neurodegenerative diseases.

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Application Of SPECT/CT Imaging System For Investigation Of Blood Kinetics And Tissue Distribution Of Radiolabeled Plumbagin In Healthy And Plasmodium Berghei-Infected Mice

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Introduction. Plumbagin is a derivative of napthoquinone that extract from roots of plants in several families. The compound exhibits a wide range of biological and pharmacological activities including antimalarial, antibacterial, antifungal, and anticancer activities.
Aims. The present study was to investigate blood kinetics and tissue distribution of plumbagin in healthy and *P. berghei*-infected mice using SPECT/CT.
Methods. Plumbagin was labelled with ^{99m}technetium and stannous chloride dihydrate (reducing agent) under various conditions and pH and the labelling yield was optimized. Blood kinetics and biodistribution of ^{99m}Tc-plumbagin complex was investigated using gamma counter and SPECT/CT imaging system in healthy and *P.burghei*-infected mice model.
Results. *In vitro* and *in vivo* studies suggested satisfactory stability profiles of ^{99m}Tc-plumbagin complex in serum and normal saline (92.21 to 95.47%) within 24 h. Significant difference in blood kinetics (mean residence time, systemic clearance and apparent volume of distribution) was observed between *P. berghei*-infected and healthy mice. The labelled complex distributed to all organs of both healthy and infected mice but with high intensity in liver, followed by lung, stomach, large intestine, and kidney. Accumulation in spleen was markedly noticeable in the infected mice.
Discussion. SPECT/CT imaging with radiolabelled ^{99m}Tc is a viable noninvasive technique that can be applied for investigation of kinetics and biodistribution of plumbagin in animal models. Plumbagin-labelled complex was rapidly cleared from blood and major routes of excretion were hepatobiliary and pulmonary routes. Malaria disease state influenced the pharmacokinetics and disposition of plumbagin.

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Evaluation of Modafinil as a Perpetrator of Clinically Relevant Metabolic Drug-Drug Interactions

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Introduction. Modafinil is a novel vigilance promoting (eugeroic) drug with a rapidly expanding range of indications and hence increasing potential for co-administration with alternate medications. The capacity of modafinil to perpetrate metabolic drug-drug interactions (MDDIs) has been demonstrated *in vitro* for a panel of drug metabolising cytochrome P450 (CYP) enzymes. However, the magnitude and clinical relevance of these *in vitro* data remains to be demonstrated. Given the increasing use of modafinil, the capacity for this drug to perpetrate clinically relevant MDDIs requires clarification.
Aim. Evaluate the magnitude and clinical relevance of MDDIs perpetrated by modafinil using an integrated physiological based pharmacokinetic (PB-PK) modelling and clinical study approach.
Methods. PB-PK simulations were undertaken in virtual healthy volunteers (n=120) using SimCYP. A modafinil inhibitor profile was created based on this drugs physiochemical properties and published *in vitro* interaction data for CYP1A2, 2C19, 2D6 and 3A4. Virtual trials were performed to assess the impact of a single dose of modafinil (200mg; PO) and steady state dosing of modafinil (200mg; PO for 7 days) on the CYP selective probes caffeine (CYP1A2), omeprazole (CYP2C19), dextromethorphan (CYP2D6) and midazolam (CYP3A). A confirmatory single centre, open label, cocktail drug interaction study in health volunteers (n=12; MODDI-14; ACTRN12614000451606) is currently underway.
Results. Following a single 200mg oral dose of modafinil the mean simulated area under the plasma concentration–time curve (AUC) ratios for caffeine, omeprazole, dextromethorphan and midazolam were 1.00, 1.14, 1.00 and 0.98, respectively. Following dosing of modafinil to steady state (200mg for 7 days), the simulated AUC ratios for caffeine, omeprazole dextromethorphan and midazolam were 0.98, 1.14, 1.00 and 0.71, respectively.
Discussion. PB-PK simulations demonstrate that, when dosed to steady state, modafinil may perpetrate clinically relevant MDDIs with CYP3A. This interaction will be confirmed in the MODDI-14 clinical study.

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Targeting Arginine Metabolomics in Older Individuals

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Introduction. Nitric oxide synthase (NOS) mediated conversion of arginine (ARG) to citrulline (CIT) is a key pathway for the synthesis of nitric oxide, which has important homeostatic and pathophysiological roles in several age-associated diseases. However, ARG is also metabolised by alternate pathways involving conversion to ornithine (ORN), homoarginine (HMA), N^G-monomethyl-L-arginine (MMA), N^G,N^G-dimethyl-L-arginine (ADMA) and N^G,N'^G-dimethyl-L-arginine (SDMA), all of which alter NOS activity.
Aim. Establish a comprehensive ARG metabolomics platform to assess age-associated diseases.
Methods. Plasma samples were obtained from a cohort of 125 healthy older (age >60years) individuals from the Hunter Community Study. Analytes (ARG, ADMA, SDMA, MMA, HMA, CIT and ORN) were isolated by solvent extraction, evaporated to dryness and reconstituted. Separation of analytes was achieved by ultra-performance liquid chromatography (UPLC) using a gradient mobile phase comprising ammonium formate (10mM, pH 3.8) in water and methanol (1% to 63%). Analytes were detected by quadrupole time-of-flight tandem mass spectrometry (Q-ToF-MS) operated in positive ion mode with electrospray ionization (ESI^+). Data were collected using MS^E technology.
Results. Solvent extraction provided consistent, high recovery (>95%). UPLC-QToF-MSE facilitated the separation and quantification of the 7 chemically similar analytes in a total analysis time of 6min. The approach had high sensitivity; LOQ range from 0.005µM (NMMA) to 0.25µM (ARG and ORN), and good precision; intra- and inter-day %RSD <6% for all analytes, all of which are fit-for-purpose. Mean (±S.D) plasma analyte concentrations (µmol/L) were: ARG 86.1 (±29.8), ADMA 0.55 (±0.07), SDMA 0.77 (±0.15), NMMA 0.09 (±0.03), HMA 0.98 (±0.35), CIT 27.2 (±8.7) and ORN 62.7 (±24.2).
Discussion. This study describes a robust, yet rapid, analytical approach to determine a comprehensive panel of ARG metabolites, which can be used to assess the involvement of ARG metabolism in age-associated diseases at a population level and identify potential novel drug targets within this pathway.

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Chemical constituents of AVS022 a polyherbal formula and its herbal components by Liquid Chromatography Quadrupole Time-of-flight Mass Spectrometry

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Introduction. Ayurved Siriraj Ha-Rak Recipe (AVS022), a Thai traditional polyherbal formula has been widely used as an antipyretic drug in Thailand. AVS022 is prepared by the combination of five dried roots powder in the equal parts by weight; *Capparis micracantha* DC., *Clerodendrum petasites* (Lour.) S. Moore., *Harrisonia perforata* (Blanco) Merr, *Ficus racemosa* Linn. and *Tiliacora triandra* (Colebr) Diels. Previous scientific studies have suggested that AVS022 had the antioxidant (1) and anti-inflammatory (2) effects. However, it's lack of scientific data showing their chemical constituents.
Aims. To isolate and identify of chemical compounds from AVS022 and its five herbal components.
Methods. Methanol extracted of AVS022 and its five herbal components were separated by LC-QTOF. Chemical compounds were identified by comparison of empirical molecular formula, isotope pattern matching and fragmentation pattern matching with online database.
Results. Nineteen chemical constituents were found from positive and negative electrospray ionization (ESI) mode.
Discussion. This work developed the first chemical profiling of AVS022 and its five herbals component by LC-QTOF. The method has been developed for "non-targeted" profiling of AVS022 and its herbal components. Nineteen chemical constituents included flavonoid and the other group such as hesperitine and iso-Corydine which were known as pharmacological substances of antioxidant and anticancer activity were found from positive and negative ESI mode.

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Information Seeking Behaviors of Breastfeeding Women When Considering the use of Over-The-Counter Medicines

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INTRODUCTION. Breastfeeding women is a population that will often require an over-the-counter medicine. Safety of medications during breastfeeding can be concerning to both breastfeeding women and health professionals. Information regarding the safety of medications during breastfeeding is often limited and/or conflicting which may lead to confusion or inappropriate health advice.
AIM. To understand the information seeking behaviors of breastfeeding women, the type of information sought, trusted information sources, preferred form(s) to receive information and how easily it is understood.
METHODS. A pilot survey was implemented within an Australian community pharmacy setting. Women were either recruited by pharmacy staff or by the baby care clinic nurse.
RESULTS. This study of 23 women found breastfeeding women actively seek information about over-the-counter medicines prior to their use during breastfeeding. Information is often sought from various healthcare professionals and multiple sources are used prior to the decision to use a medication. Network analysis showed that women preferred to source information (in descending order) from a pharmacist, general practitioner, the internet, and a nurse or lactation consultant. Peer groups were less frequently used to source drug safety information. The majority of women reported that verbal information was easier to understand than written information.
DISCUSSION. Understanding information seeking behaviors of breastfeeding women can drive improvements in provision of advice and support. For example, this pilot study suggests that improvements could be made in written drug safety information intended for consumers. Extension of this study can provide a greater understanding of these behaviors in the wider population with the ultimate goal of improving health literacy for breastfeeding women.

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A Study of The Effects of *Andrographis paniculata* On Platelet Aggregation In Thai Healthy Volunteers: A Pilot Study

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Introduction. *Andrographis paniculata* (Burm.F) Wall ex Nees (AP) have long been used in Thai traditional medicine to relieve the common cold, fever, and diarrhea. The effects of AP on human platelet function have not been reported .
Aims. To study the effects of AP on platelet activity in healthy human volunteers.
Methods. Blood was taken before and after AP ingestion 2 and 24 hours later. Platelet aggregation induced by ADP, epinephrine and collagen was studied using aggregometer. COX mRNA and protein expression in platelets were studied using real time PCR and Western blot while P-selectin was also studied using EIA.
Results. Heterogeneity of the effects of AP on platelet function was shown. AP significantly decreased ADP-induced platelet aggregation whereas it increased epinephrine-induced aggregation. COX-1 mRNA, but not protein expression, was significantly increased. Interestingly, COX-2 mRNA and protein was also detected. However, P-selectin was not significantly changed.
Discussion. This is the first preclinical trial of AP effects on human platelet aggregation. The effects of AP on platelet function were shown to be multifactorial.

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An Exploratory Study Of The Dietary Patterns Of North East Asian Subjects Participating In An Australian Phase I Clinical Study

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Introduction. To support drug development in North East Asia (NEA) Phase I clinical studies are frequently conducted in healthy Japanese, Chinese and Korean subjects residing in the West, including in Australia. It is not known whether the dietary patterns of these subjects, which could potentially influence drug pharmacokinetics, reflect their native country or their new environment in the West.
Aim. To explore the dietary patterns of healthy NEA subjects participating in a Phase I study in Sydney.
Methods. Diet diaries were collected from healthy Chinese (n=20), Japanese (n=19) and Korean (n=20) subjects participating in a Phase I study in Sydney. Ethics committee approval was obtained and all subjects provided written informed consent. The meals consumed by each subject over 9-12 days were recorded. Each meal was classified based on relevant literature as Traditional to their ethnic group, Western, Mixed, Neutral or Other Foreign and the frequency of consumption determined. Ethnic-specific foods with the potential to influence pharmacokinetics (e.g. kimchi, green tea) were also examined.
Results. Meals classified as Traditional were the most frequently consumed meals for the Chinese (mean ± SD, 9.5 ± 3.1 meals/week), Japanese (7.4 ± 3.5 meals/week) and Korean (10.5 ± 4.6 meals/week) subjects. The Chinese, Japanese and Korean subjects also consumed a substantial number of Western meals (4.9 ± 3.6, 6.8 ± 3.7 and 5.9 ± 3.8 meals/week, respectively). A lower consumption of the remaining meal types was reported. For all three ethnic groups, Western meals were predominantly consumed at breakfast, as is reported in NEA. Traditional meals were more commonly consumed at lunch and dinner. Kimchi was consumed by 90% of Korean subjects and green tea by 16% of the Japanese subjects.
Discussion. The healthy NEA Phase I subjects in Sydney retained a predominantly NEA diet. Breakfast was the most westernised meal of the day. These observations support the relevance of Phase I data collected in NEA subjects in Sydney to the respective populations in NEA.

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Metformin Suppresses the Pro-Metastatic Protein, CXCR4, Through HER2 Inhibition

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Introduction: Metformin (1,1-dimethylbiguanide hydrochloride), an anti-hyperglycemic drug, has been associated with reduced risk of cancer in diabetics. *In vitro* and *in vivo* studies in a variety of cancer cell lines have presented results consistent with these observations. Metformin has been shown to down-regulate the human epidermal growth factor receptor 2 (HER2), a cell surface receptor over-expressed in 30% of breast cancers. Of particular interest, HER2 upregulated the expression of another pro-metastatic receptor, the CXC chemokine receptor 4 (CXCR4), which is the most common chemokine receptor expressed in cancer cells. HER2 is known to up-regulate CXCR4 expression through the PI3K/Akt/mTOR pathway.
Aim: The aim of our study was to investigate whether metformin is able to suppress CXCR4 activity through inhibition of HER2.
Methods: Cell cytotoxicity assay and flow cytometry analysis were performed to study the anti-proliferative effects of metformin on two HER2-positive breast cancer cell lines, BT-474 and SKBr3. Levels of HER2, CXCR4 and proteins involved in the regulation of CXCR4 activity and the cell cycle were determined by western blot studies. Finally, cell migration and invasion experiments were conducted to investigate the effects of metformin on CXCR4 at the functional level.
Results: Our study demonstrated over-expression of HER2 and CXCR4 in both breast cancer cell lines. Metformin-induced G1/S cell cycle arrest in BT-474. Levels of CXCR4 were suppressed by metformin and associated with simultaneous decreases in levels of HER2, PI3K, p-Akt and p-mTOR. At the functional level, metformin was found to inhibit CXCR4-mediated cell migration in SKBr3 and cell invasion in both BT-474 and SKBr3 cell lines.
Discussion. The results are consistent with the hypothesis that metformin suppresses CXCR4 levels through inhibition of the PI3K/Akt/mTOR pathway initiated by HER2. These findings suggest a novel property of metformin of inhibiting a pro-metastatic protein in HER2-positive cancer cell lines.

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Deprescribing In Australian Residential Aged Care Facilities: What's Do GPs, Nurses, Pharmacists And Residents Consider Important?

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Introduction. Polypharmacy and multimorbidity are common among older people in residential aged care facilities (RACFs). Polypharmacy has been associated with increased hospitalisations, adverse drug events, drug interactions, falls, impaired functional capacity and cognitive impairment. Reducing polypharmacy may reduce adverse events and improve resident's quality of life. Deprescribing refers to cessation of medications after consideration of therapeutic goals, benefits and risks, and medical ethics.
Aims. To rank factors which general practitioners (GPs), nurses, pharmacists and residents perceive are most important when deciding whether or not medications should be deprescribed.
Methods. Discipline-specific groups of GPs (n=13), nurses (n=6), pharmacists (n=9) and residents/representatives (n=6) associated with RACFs were conducted in South Australia. Nominal group technique was used to discuss, explore and rank factors each discipline perceived as important when deciding whether or not to deprescribe medications.
Results. Participants identified a wide range of factors with considerable overlap between disciplines, however no two disciplines had the same priorities. The highest ranked factors for each discipline were:
GPs – evidence for deprescribing; communication with family/resident
Nurses – GP receptivity to deprescribing; nurses ability to advocate for residents;
Pharmacists – clinical appropriateness of therapy for individual residents; identifying a resident's goal of care;
Residents – residents wellbeing; poor continuity of nursing staff.
Discussion. Multiple factors that influence deprescribing decisions in RACFs were identified, with each discipline having different priorities. The factors important to each discipline need to be considered in the design of deprescribing interventions in this setting.

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What Factors Do Multidisciplinary Care Teams Consider Important When Deprescribing In Australian Residential Aged Care Facilities?

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Introduction. Polypharmacy and multimorbidity are common among older people in residential aged care facilities (RACFs). Polypharmacy has been associated with increased hospitalisations, adverse drug events, drug interactions, and cognitive impairment. Reducing polypharmacy may reduce adverse events and improve resident's quality of life. Deprescribing refers to cessation of medications after consideration of therapeutic goals, benefits and risks, and medical ethics.
Aims. To rank factors which metropolitan and regional multidisciplinary groups (comprising general practitioners (GPs), nurses, pharmacists and residents' representatives) consider to be most important when deciding whether or not medications should be deprescribed in the RACF setting.
Methods: Multidisciplinary groups were convened in metropolitan and regional South Australia. Using nominal group technique, the groups discussed, explored and ranked factors they perceived important for deprescribing.
Results. The metropolitan group ranked 'adequacy of a resident's medical and medication history' as the most important factor. The regional group ranked 'identifying a resident's goal of care' the most important factor. Both metropolitan and regional groups ranked the 'structure of the health system' as an important factor that impacts their decision to deprescribe. Both groups identified factors relating to interdisciplinary cohesiveness and communication as important, with the metropolitan group ranking them highly.
Discussion. Patient-centred factors were the most important overall. However, the structure of the health system had a considerable impact on the decision to deprescribe medications. Metropolitan and regional multidisciplinary groups prioritised different factors. This suggests that for deprescribing to be effective, local factors should be taken into account while implementing a patient-centred, multifaceted approach.

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Two Blood Test Parameters, RDW And AST May Predict High Serum Valproic Acid Levels İn Epileptic Patients

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Introduction.Text Valproic acid is one of the oldest but still widely used anticonvulsant drugs. Serum level of valproic acid is monitored to achieve the best therapeutic potency with minimal side effects. However therapeutic drug monitoring is a less accessible test compared to routine blood tests.
Aims. The present study was designed to investigate the most important hematological and biochemical indicators of high serum valproic acid levels.
Methods.The patients who underwent valproic acid level estimation in 2013 were drawn from the database of the local hospital and the patients with high and borderline high valprioc acid levels (>90 mg/L) were selected. The 31 data points from 23 patients were analyzed. The corresponding hematological data, including complete blood count and blood chemistry tests, conducted on the same day of the valproic acid level measurement were evaluated by a multiple regression analysis.
Results.None of the single parameters evaluated was significantly correlated with serum valproic acid level. However a backward stepwise regression analysis revealed that the red blood cell distribution width (RDW) (ß = -0.39) together with aspartate aminotransferase (AST, SGOT) (ß = 0.313) were the main predictors of serum high valproic acid levels in patients [$F(2,24) = 3.483$; $p < 0.05$].
Discussion.In the patients on valprioc acid therapy, none of the parameters directly reflects increased blood levels of valprioc acid. However, in clinically suspected patients, a decreased RDW value together with an increased AST levels should be taken into consideration for an increase in serum valproic acid level.

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Nrf2-mediated Inhibition of Neuroinflammation by Tiliroside

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Introduction. Excessive production of ROS is invoved in oxidative stress mediated neuroinflammation. It has been indicated that activation of Nrf2 signaling attenuates oxidative stress in neuroinflammation. Our previous study showed that tiliroside inhibited LPS/IFNγ-activated neuroinflammation in BV2 microglia by inhibiting NF-κB activation and inducing Nrf2/HO-1 activation (1).
Aim. To investigate whether anti-neuroinflammatory action of tiliroside is mediated through Nrf2 signalling in LPS/IFNγ-activated BV2 microglia.
Methods. Cultured BV2 cells were pre-treated with tiliroside (6μM) prior to stimulation with LPS (100 ng/ml) /IFNγ (5 ng/ml) for 24 h, supernatants were evaluated for the levels of nitrite, PGE_2 and TNFα. BV2 cells were transfected with Nrf2 siRNA. Western blotting was used to evaluate the protein expressions of iNOS and COX-2.
Results. Pretreatment with tiliroside (6μM) significantly ($p<0.001$) reduced LPS/IFNγ-stimulated nitrite, PGE_2 and TNFα production in BV2 microglia. However in Nrf2 knockout cells, this reduction was singinificantly ($p<0.01$) reversed. Also, LPS/IFNγ-activated iNOS and COX-2 protein expressions were significantly ($p<0.0001$) downregulated by tiliroside (6μM), which was reversed in Nrf2 knockout cells.
Discussion. Taken together, our results showed that tiliroside inhibits LPS/IFNγ-activated neuroinflammation in BV2 microglia via the Nrf2 signalling pathway.

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Determination of Aristolochic acid I in Ayurved Siriraj Ammareutavatee recipe using Solid Phase Extraction and HPLC

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Introduction. The widespread occurrence of Aristolochic acid (AA) toxicity in Aristolochia plants such as *Aristolochia tagala* Cham (Krai-krue; KK) cause greatly restricted their usefulness as dietary supplements or medicines. Ayurved Siriraj Ammareutavatee (AMT), a Thai traditional herbal recipe, contain KK.
Aim. To determine Aristolochic acid I (AA-I) in AMT.
Methods. AMT were used for detection of AA-I by HPLC technique. Each batch of AMT comprise of 3 sample types, AMT (contain KK), AMT without KK, and KK. All samples were extracted using 2 techniques, sonication and solid phase extraction (SPE). After that, extractants were injected into HPLC with an appropriate condition.
Results. No AA-I found in AMT without KK. The amount of AA-I in AMT and KK by sonication are 6.78±0.05 and 8.02±0.08 μg/mL, whereas the amount in SPE is 9.81±0.12 and 11.34±0.11 μg/mL.
Discussion. AA-I were not found in AMT without KK. Lead to the hypothesis that, in this recipe the detected AA-I are come from only KK component. SPE show valuable advantage of cleaning up the contaminate peak in AMT batch 2, resulting in determination of the amount of AA-I in this batch.

(1) Yamasaki K (2009) J Nat Med. Oct;63(4):451-8

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KMUP-1 reduces neuropathic pain via PKA and PKC signaling in dorsal root ganglion

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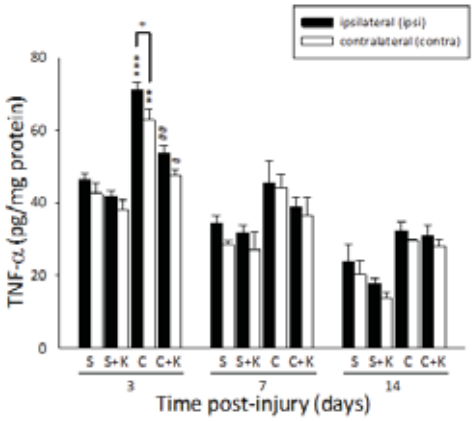
Introduction. Neuropathic pain is a refractory pain characterized by its complex mechanisms and diverse clinical manifestations. Traditional therapies usually bring about many side effects and limited success.
Aims. To investigate whether KMUP-1 could reduce hyperalgesia and inflammatory pain and to reveal its mechanisms in dorsal root ganglion (DRG) following chronic constriction injury (CCI)-induced neuropathic pain.
Methods. Sprague–Dawley rats were divided into four groups: sham, sham+KMUP-1, CCI and CCI+KMUP-1. KMUP-1 (5 mg/kg, i.p.) was administrated once daily starting at day 1 after CCI surgery. Each group of rats (n=6) were sacrificed and L4-L6 DRGs removed quickly at day 3, 7 and 14 after CCI.



Results. KMUP-1 decreased mechanical allodynia at day 3, 7 and 14, and thermal hyperalgesia at day 7 and 14 after CCI ipsilateral side, but not CCI contralateral side. KMUP-1-treated group significantly inhibited CCI-induced inflammatory mediators (iNOS, COX2) and proinflammatory mediators (TNF-α, IL-1β). Activation of PKA, PKC and ERK in the DRG contributes to the initiation of CCI-induced pain hypersensitivity. KMUP-1 also inhibited the PKA, PKC and ERK activations that could partly attribute to its possible mechanisms in CCI-induced inflammatory pain.
Conclusion. KMUP-1 has anti-inflammation and anti-hyperalgesia in CCI-induced pain behaviors via inhibition of PKA, PKC and ERK, suggesting that it can be a potential candidate for neuropathic pain.

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MicroRNAs As Biomarkers For Alzheimer's Disease – A Systematic Review And Meta-analysis

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Introduction. Alzheimer's disease (AD) is a major source of disease burden worldwide, however currently there are no effective treatments. Barriers to the development of effective therapy include the lack of a specific and easily accessible biomarker for the early identification of disease. In recent years, microRNAs (miRNA), a class of non-coding RNA known to regulate protein expression post-transcriptionally, have been recognised as novel biomarkers for diseases.
Aims. To evaluate systematically the literature on the potential use of circulating miRNAs as a biomarker for diagnosing AD.
Methods. Eligible studies of miRNAs in peripheral blood distinguishing AD or mild cognitive impairment (MCI) from cognitively normal controls were identified through standardised search strategies in Medline, Pubmed and Embase, and assessed for relevance and quality. Results were pooled using random-effects models to summarise the overall test performance of miRNAs.
Results. Thirteen studies, covering 67 types of miRNA and involving 766 AD patients, 88 MCI patients and 877 normal controls, investigated the diagnostic value of miRNAs as a peripheral biomarker for AD. The pooled sensitivity and specificity were 0.84 (95% CI 0.80-0.87) and 0.73 (0.69-0.77) respectively. The pooled diagnostic odds ratio was 21.69 (10.89-43.20). A meta-analysis of the summary receiver operating characteristic (SROC) curve for the diagnostic accuracy of miRNAs in AD showed the area under the curve was 0.8930.
Discussion. The results suggest that miRNAs are relatively accurate in diagnosing AD and show potential as biomarkers. Further studies with standardised study designs are required to validate these results before miRNAs can be applied clinically. Insight into factors that influence miRNA expression such as genetic variation and environmental factors from different populations is also required especially if miRNAs are to inform future patient selection for therapy and drug trials.