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**Omega-3 fatty acids modulate Weibel-Palade body degranulation and actin cytoskeleton rearrangement in PMA-stimulated human umbilical vein endothelial cells**

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Introduction. Von Willebrand factor (vWF), released from endothelial storage granules (Weibel-Palade bodies; WPBs), is thrombogenic (Ruggeri, 2007). Although anti-thrombotic effects have been ascribed to long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs), their mechanisms of action have not been fully investigated.
Aims. To determine whether LC n-3 PUFAs regulate WPB degranulation in stimulated human cultured umbilical vein endothelial cells (HUVECs).
Methods. HUVECs were incubated with or without 75 or 120 $\mu$M docosahexaenoic acid or eicosapentaenoic acid for 5 days at 37°C. WPB degranulation was stimulated using phorbol 12-myristate 13-acetate (PMA), and this was assessed by immunocytochemical staining for vWF. Actin reorganisation was determined using phalloidin-TRITC staining.
Results. We found that PMA stimulated WPB degranulation, and that this was reduced by prior incubation of cells with 120 μM LC n-3 PUFAs (control: 75.7±6.7%, PMA: 5.7±1.5%, PMA+DHA: 20.7±1.3%, PMA+EPA: 17±5.9% of granulated HUVECs, one-way ANOVA, n=3, $p<0.05$). In these cells, WPBs had rounded rather than rod-shaped morphology and localised to the perinuclear region, suggesting interference with cytoskeletal remodelling that is necessary for complete WPB degranulation. In line with this, actin rearrangement was altered in cells containing perinuclear WPBs, where cells exhibited a thickened actin rim in the absence of prominent cytoplasmic stress fibres.
Discussion. These findings indicate that LC n-3 PUFAs provide some protection against WBP degranulation, and may contribute to an improved understanding of the anti-thrombotic effects previously attributed to LC n-3 PUFAs.

Ruggeri ZM (2007) Thromb Res 120:S5-S9.

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**Dissociation between proportion of perhexiline assays within therapeutic range and clinical demographics or steady-state pharmacokinetics during long-term therapy**

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Introduction. The purpose of therapeutic drug monitoring is to ensure that while drug efficacy is maintained, adverse events are minimised. However, in clinical setting, it is often unknown if therapeutic target is achieved most of the time. In the case of cardioprotective agent perhexiline (Px) with complex pharmacokinetics, it is recommended that 3-monthly drug level be assessed to ensure its efficacy.
Aims. As a component of an evaluation of the long-term safety of Px therapy, we assessed (1) the relationship between frequency of drug monitoring and proportion of therapeutic levels (PTL); and (2) the determinants of PTL.
Methods. Retrospective analysis was performed on patients who were administered Px for at least twelve months between 1997-2003. Determinants of PTL were sought utilizing backward stepwise multiple linear regression.
Results. 95 patients, aged 71 ± 11.3 (SD) were included in the analyses. Their median (range) duration of treatment was 53 months (27, 96). Majority of patients (62%) had apparent at least 3-monthly monitoring, which is the current recommended monitoring frequency. 18% had 3-6 monthly monitoring, and up to 20% had less than 6-monthly monitoring. The median (range) of PTL, subtherapeutic levels and supratherapeutic levels were 63% (50,77), 23% (11, 42) and 5% (0, 13) respectively. PTL displayed a significant linear correlation with duration of therapy ($r^2$ = 0.08, $p<0.05$). However, there was no significant correlation found between PTL and the following parameters: age, weight, frequency of monitoring, dosage, ejection fraction and duration of therapy. Multivariate analyses performed to identify parameter that might affect PTL, again showed no significant correlation.
Discussion. While there is no significant determinant of PTL on a multivariate analysis at this stage, it is likely that concomitant disease states, such as congestive heart failure, which are associated with episodic non-compliance, may imperil drug utility.

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**Lower dose statin pharmacotherapy may be sufficient and safer**

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Introduction. Statins are regarded as essential in coronary disease but more than 15% of patients experience significant side effects, such as myopathy, fatigue, cognitive blunting, neuropathy, hepatitis, renal failure and cataracts, some dose related. The recommended starting dose of atorvastatin in the NICE guidelines is 10mg, which reduces LDL-cholesterol by 1.8 mmol/L and coronary risk by more than 60%, after 3 years. In the TNT study, patients sustained 22% less recurrent coronary events with 80, compared to 10 mg, of atorvastatin daily, but with a 6-fold increase in liver toxicity.
Aims. To evaluate statin dosing in the community.
Methods. Statin prescribing was audited for 1 month at a busy metropolitan Pharmacy.
Results. Most prescriptions were for rosuvastatin or atorvastatin, one half at doses well above the NICE guideline starting dose. The statin dose prescribed varied 32-fold.

Statin Doses Prescribed at a Perth Pharmacy
(Dispensed 1 July - 31 July 2013)

| | 5mg | 10mg | 20mg | 40mg | 80mg | Total | Mean Dose (mg)* | Suggested Dose (mg) | Range of Dose |
|---|---|---|---|---|---|---|---|---|---|
| Pravastatin | | 0 | 0 | 4 | 0 | 4 | 40 | 80 | - |
| Simvastatin | 0 | 2 | 11 | 8 | 2 | 23 | 31 | 40 | 8 fold |
| Atorvastatin | | 8 | 16 | 14 | 10 | 48 | 37 | 10 | 8 fold |
| Rosuvastatin | 6 | 24 | 14 | 9 | | 53 | 17 | 5 | 8 fold |
| | | | | | Total | 128 | | | |

*rounded to a whole number

Discussion. Higher doses may be favoured in symptomatic coronary disease, lower doses because of side effects. Earlier landmark trials showed substantial reductions in coronary events with 20-40mg of simvastatin (4S, HPS) or 40mg of pravastatin (WOSCOPS, Lipid) daily and it may be that atorvastatin and rosuvastatin, with their much longer half-lives and broadly 8 times greater potency, are prescribed at similar doses out of habit. Serum cholesterol levels are not a reliable basis for dose titration because they are often confounded by diet, posture and intercurrent illness. It may be more appropriate to individualise statin dose on the basis of the severity of patient's coronary disease, which can now be readily evaluated non-invasively with computed tomography. Most clinicians prescribe very uniform doses of other atheroma treatments, for example aspirin (100mg) or irbesartan (150mg). Rather than increasing the statin dose unnecessarily, closer attention to reduction of weight, smoking, blood pressure and blood glucose is likely to reduce coronary risk more efficiently.

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**The influence of the fibrotic microenvironment on glucocorticoids sensitivity in lung fibroblasts.**

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Introduction. Idiopathic pulmonary fibrosis (IPF) is a progressive, fibrotic lung disease. Lung fibroblasts are key contributors to IPF pathology, producing excessive extracellular matrix (ECM) components and profibrotic mediators, such as TGF-β. Fibroblasts are considered a key drug target in IPF. Glucocorticoids may be beneficial considering their pleiotropic effects on airway structural cells, such as impacting on TGF-beta production in fibroblasts, and reducing mesenchymal cell proliferation. However, IPF is a glucocorticoid-resistant condition. Considering the lack of efficacy of other pharmacological IPF treatments, an improved understanding of glucocorticoid resistance mechanisms in IPF remains an imperative. The fibrotic microenvironment may impact on glucocorticoid sensitivity in airway structural cells. TGF-β induces glucocorticoid insensitivity in bronchial epithelial cells (Salem et al, 2012), and culturing airway smooth muscle cells (ASM) on type I collagen renders ASM insensitive to the anti-proliferative effects of glucocorticoids (Bonacci et al, 2006).
Aims. To ascertain the influence of the fibrotic microenvironment on glucocorticoid sensitivity in lung fibroblasts.
Methods. Primary fibrotic lung fibroblasts were seeded on plastic or type I collagen at 20,000 cells/well for 72h. RT-qPCR was performed on mRNA extracted from cells treated for 24h with 100pM TGF-β then 2h with 100nM dexamethasone.
Results. TGF-β enhanced the dexamethasone-induced increase in the glucocorticoid-regulated gene, glucocorticoid-induced leucine zipper (GILZ), by 12.0±1.5-fold (n=6; $P<0.001$) compared to dexamethasone alone. TGF-β increased glucocorticoid receptor (GR)-α transcript levels by 2.5±0.3-fold (n=6; $P<0.001$).
Discussion. In contrast to findings in ASM and bronchial epithelial cells, TGF-β does not impair and may enhance glucocorticoid responsiveness in lung fibroblasts.

Bonacci J et al (2006) Br J Pharmacol 149:36-373
Salem S et al (2012) Br J Pharmacol 66: 2036-2048

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**Taste GPCR functionality and contractile effects in human hearts**

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Introduction. The sensation of taste is mediated by G protein-coupled receptors and has developed as a regulator of feeding behaviour in many species, including humans. Recent studies have shown that taste receptors are expressed in a variety of tissues, including the heart. Our hypothesis is that taste receptors contribute to cardiac physiology.
Aims. To investigate whether bitter taste ligands activate their cognate bitter taste receptors (TAS2Rs) in human right atrium to modulate cardiac contractility.
Methods. Right atrial appendages were surgically removed from patients undergoing coronary artery bypass grafts and/or aortic valve replacement at The Prince Charles Hospital. Intact right atrial trabeculae were dissected, mounted onto tissue electrode blocks and electrically paced in a 50 mL organ bath. An initial screen using bitter ligands (1 mmol/L) with known TAS2R targets was performed in human heart. Drugs that had a significant contractile effect were then tested at lower concentrations. Receptor-dependent ligand responses were validated in a heterologous expression system using a fluorescent imaging plate reader (FLIPR).
Results. Several bitter ligands tested, including chloroquine, flufenamic acid, picrotoxinin, and andrographolide caused robust cardiodepressive effects on the right atrial tissue (93, 86, 73, 81 ± 5% reduction in cardiac contractility, respectively, $n \geq 5$ hearts). These effects were concentration-dependent. All tissues responded to 200 $\mu$mol/L isoprenaline. Interestingly, chloroquine also caused an increase in relaxation phase time. In addition, these bitter ligands activated their cognate TAS2Rs *in vitro*.
Conclusion. Our findings suggest that bitter taste receptors are playing a functional role in calcium mobility within cardiac tissues, as well as potentially interfering with the diastolic phase during contraction. This project highlights a new area of cardiovascular biology and drug discovery and identifies new ligands and receptors that may be utilised in modulating cardiac function.

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**TGFβ-induced non-canonical pathway regulates Nox4 expression and proliferation of endothelial cells**

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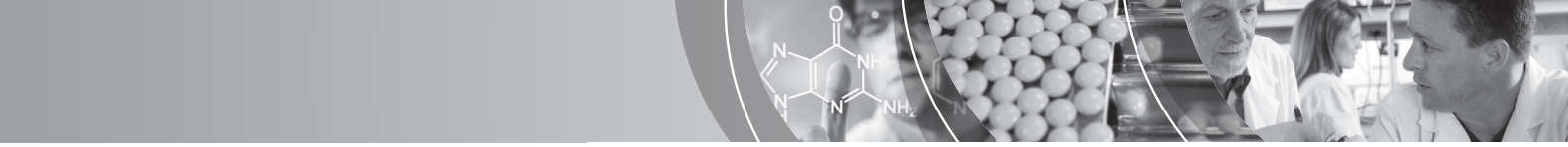
Introduction: NADPH oxidase-derived reactive oxygen species are important for various cellular functions including proliferation. Vascular cells predominantly express Nox4 isoforms of NADPH oxidase, however, its regulation in endothelial cells is not clear yet.
Aims: In this study, we investigated the signaling pathways involved in transforming growth factor-β (TGF-β)-induced Nox4 expression and proliferation of human microvascular endothelial cells (HMECs).
Methods: Nox4 gene expression and cell signalling pathways were measured by real time PCR and Western blot analysis in HMECs respectively. Endothelial cell proliferation was measured by DNA content based CyQUANT® NF Cell Proliferation assay.
Results: Nox4 mRNA level (but not Nox2), was significantly ($n=4$, $p<0.01$) increased by TGF-β in a time- and concentration-dependent manner. Acute treatment of HMECs with TGF-β enhanced the phosphorylation of Smad2 and extracellular signal-regulated kinase (ERK) 1/2, without effects on p38MAPK, Akt or JNK1/2 pathways. Inhibition of activin receptor-linked kinase 5(ALK5) with SB431542 reduced TGF-β -induced Nox4 mRNA expression whilst inhibition of ERK with U0126 decreased basal Nox4 mRNA expression. Inhibition of Smad2 phosphorylation with SB431542 did not affect ERK 1/2 phosphorylation whilst ERK 1/2 inhibition with U0126 did not affect Smad2 phosphorylation. Finally, addition of TGF-β enhanced endothelial cell proliferation and is reduced by ERK1/2 inhibitor U0126 but not by ALK5 inhibitor SB431542.
Discussion: These findings suggest that TGF-β-induced Nox4 via non-canonical pathway is important for the proliferation of endothelial cells which is vital during angiogenesis.



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**Hydrogen sulfide as a vasorelaxant in mouse mesenteric arteries**.
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Introduction: Hydrogen sulfide is a gaseous mediator that has vasoactive effects.
Aims:This study examines the mechanism of $H_2S$-induced vasorelaxation in resistance-like arteries and the contribution of endogenously released $H_2S$ to regulation of vascular tone.
Methods: Mesenteric arteries from C57 and $eNOS^{-/-}$ mice were mounted in small vessel myographs for *in vitro* recording of isometric force. Expression of the $H_2S$-producing enzyme, cystathionine-γ-lyase (CSE) in mouse mesenteric arteries was also examined.
Results: NaHS elicited a biphasic vasorelaxation response in C57Bl6/J mouse mesenteric artery. The $1^{st}$ phase of the relaxation response was endothelium-dependent, absent in arteries from $eNOS^{-/-}$ mice and abolished by the sGC inhibitor ODQ and high $K^+$. The $2^{nd}$ phase was $K^+$-dependent, and sensitive to inhibition of $K_V$ and $K_{Ca}$ channels. Experiments examining the contractile responses to $Ca^{2+}$ showed that NaHS can also inhibit $Ca^{2+}$ channel function. The endogenous $H_2S$-producing enzyme CSE was expressed in vascular smooth muscle and perivascular adipose cells from C57 mouse mesenteric artery. Supplying the substrate for CSE (L-cysteine) in the presence of the CSE co-factor pyridoxal 5'-phosphate caused vasorelaxation in mouse mesenteric arteries from C57Bl6/J that was sensitive to the CSE inhibitor DL-propargylglycine (PPG, 20mM). Additionally, PPG caused a concentration-dependent contraction of mesenteric artery.
Discussion: These data show that the vasorelaxant effect of $H_2S$ is complex, partly endothelium and nitric oxide dependent and sensitive to $K^+$ channel inhibition, DIDS and involves voltage-gated $Ca^{2+}$ channels. The $H_2S$ producing enzyme CSE is present in the smooth muscle and periadventitial fat of these resistance-like vessels and can be activated to cause vasorelaxation in these *in vitro* conditions. Inhibiting CSE causes an increase in vascular tone supporting a role for CSE-derived $H_2S$ in the regulation of blood vessel function.

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**Pharmacological inhibition of IL-1β signalling in DOCA/salt-induced hypertension in mice does not reduce renal inflammation or blood pressure.**
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Introduction. Renal inflammation plays a role in the pathophysiology of hypertension. Inflammasomes are signalling platforms that regulate production of pro-inflammatory cytokines such as IL-1β and IL-18.
Aims. We investigated whether hypertension in mice is associated with increased inflammasome expression in the kidneys and whether chronic treatment with anakinra, an IL-1 receptor antagonist, reduces renal inflammation and blood pressure (BP) in hypertensive mice.
Methods. Hypertension was induced in mice by uninephrectomy, subcutaneous delivery of deoxycorticosterone (DOCA) (2.4 mg per day) and replacement of drinking water with saline. Sham mice received uninephrectomy and a subcutaneous placebo pellet. At day 7 post-surgery, some mice were treated with anakinra (25 mg/kg per day, *i.p.*) or vehicle (0.9% saline, *i.p.*) for 14 days. Systolic BP was monitored via tail-cuff and, at day 21 post-surgery, mice were killed and their right kidneys removed for ex-vivo analyses. Real-time PCR analysis was used to determine mRNA expression of inflammasome subunits (NLRP3, ASC, caspase-1); inflammasome-derived cytokines (IL-1β, IL-18); and renal inflammatory markers (ICAM-1, VCAM-1, CCL5, CCL2, IL-6, IFN-γ).
Results. Systolic BP was elevated in DOCA/salt (157.1±2.8 mmHg) versus sham (122.7±3.9 mmHg) mice (n = 10, $P<0.0001$). Real-time PCR showed that mRNA expression of inflammasome subunits and inflammasome-derived cytokines were upregulated in the kidneys of DOCA/salt-treated mice (n = 9-10, $P<0.05$). Anakinra treatment tended to reduce expression of these inflammasome subunits and IL-1β, although these effects failed to reach statistical significance (n = 5-7). Similarly, anakinra had no effect on expression of renal inflammatory markers, nor did it reduce systolic BP in DOCA/salt-treated mice.
Discussion. Although hypertension in mice was associated with upregulation of inflammasome/IL-1β expression in the kidneys, anakinra had only minimal inhibitory effects on renal inflammation and systolic BP. Future studies will assess the impact of higher doses of anakinra and alternative strategies to inhibit inflammasome/IL-1β signalling.

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**Effects of dantrolene sodium and azumolene on cardiac calcium activation and CYP450 metabolism**

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Introduction. Dantrolene sodium, a skeletal muscle relaxant has been suggested as a novel cardiac drug (Maxwell et al 2012). The analogue azumolene (Sigma) may also have potential cardiac effects. Dantrolene can cause liver damage while the liver toxicity of azumolene is unknown.
Aims. To investigate the effects of dantrolene sodium and azumolene on calcium dependent cardiac contractility and cytochrome p450 metabolism in purified isoenzymes.
Methods. Cardiac contractility was measured using isolated whole snail (*Helix aspersa*) hearts exposed to varying calcium concentrations (0 to 10 mM); dantrolene and azumolene concentrations ($10^{-12}$ to$10^{-4}$M). Data were recorded using LabChart® (AD Instruments) and analysed using Graphpad®. Data are expressed as mean±SEM, and statistically analysed using the unpaired Student's t-test ($\alpha$=0.05). All experiments were conducted in accordance with ethical approval (AEC06_10BG) La Trobe University. Baculosome® assays (Invitrogen) for the CYP450 isozymes 2E1, 2C19, 3A4 and 2D6 were measured using a Flexstation3 (Molecular Devices).
Results. Dantrolene (1μM) significantly reduced (P=0.05, n=3) the $[Ca^{2+}]_o$ required for 50% activation ($EC_{50}$) but not 1$\mu$M azumolene (P>0.05, n=3). Further, dantrolene caused a dose dependent increase in contractility ($\log_{10}EC_{50}$ 9.89 n=2 observations) but azumolene did not. All CYP450 isozymes (3A4, 2C19, 2D6 and 2E1) tested were inhibited by dantrolene. Inhibition of 2D6 ($\log_{10}IC_{50}$ 5.0±0.1, n=6) was significantly different from the reference inhibitor, quinidine ($\log_{10}IC_{50}$7.9±0.1, n=6, p=7x$10^{-7}$). Azumolene inhibited CYP3A4 ($\log_{10}IC_{50}$ 6.1± 0.1, n=6) which was significantly different from the reference inhibitor, ketoconazole ($\log_{10}IC_{50}$6.9±0.1, n=6; p=0.01).
Discussion. Dantrolene significantly shifted the force-calcium relation to the left in isolated whole hearts which is consistent with it mobilising intracellular calcium stores. Dantrolene inhibited all CYP450 isozymes while azumolene only inhibited 3A4 significantly.

Maxwell J et al (2012) Am J Physiol (Heart) 302:H953-H963

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**Endothelin causes vasoconstriction of rat cerebral arteries by differential activation of voltage-operated and non-voltage-operated calcium channels**

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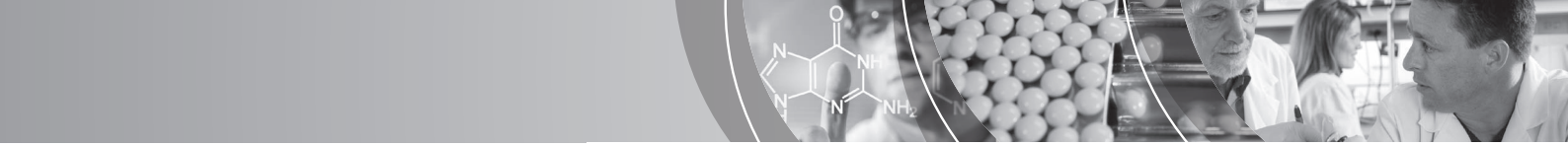
Introduction: Cerebral vasospasm is a narrowing of arteries usually occurring after subarachnoid haemorrhage (SAH) and is responsible for significant morbidities and mortalities following SAH. Endothelin-1 (ET-1) has been identified as a potential mediator in the pathogenesis of this vasospasm. This aim of this study is to analyse the role of voltage-operated calcium channels (VOCC) and non-VOCC in ET-1 induced vasoconstriction of rat cerebral arteries.
Methods: Arterial segments were dissected from different regions of the cerebral circulation and responses assessed using wire myography. ET-1 concentration-response curves were constructed in calcium-free medium or in the presence of nifedipine, NNC 55-0396 or SK&F 96365 to inhibit the L-type VOCC, T-type VOCC and non-VOCC, respectively.
Results and discussion: Neither inhibition of the calcium channels nor removal of calcium from the physiological salt solution altered the potency ($pEC_{50}$) of ET-1-induced vasoconstriction, but variable decreases in the maximum effects ET-1 (Emax) were observed. ET-1 caused a small contraction (11-22%) in calcium-free solution. Nifedipine (1 $\mu$M) significantly decreased the Emax only in the middle cerebral artery, while NNC 55-0396 (1 $\mu$M) and SK&F 96365 (30-100 $\mu$M) attenuated Emax in all cerebral arteries. Combination of nifedipine with SK&F 96365 more moderately decreased the Emax. Both nifedipine and SK&F 96365 reversed the ET-1-induced pre-contraction in all arteries, whereas NNC 55-0396 reversed the ET-1-induced pre-contraction only in posterior communicating and anterior cerebral arteries. The vasoconstriction induced by ET-1 involves differential activation of both VOCC and non-VOCC in rat cerebral arteries.



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**Advanced glycation end-products (AGE), RAGE and ROS accumulation in rat isolated arteries.**

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Introduction. Hyperglycemia leads to excessive accumulation of AGE and deleterious effects on various tissues, including blood vessels. Some of these effects are mediated by the receptor for AGE (RAGE) and signalling involving accumulation of reactive oxygen species (ROS). Most studies on RAGE signalling have been performed in isolated cells; the present study examined AGE-induced ROS signalling in intact arteries.
Aims. The aims of this study were to examine RAGE expression and the effect of glycated albumin on superoxide accumulation in arteries isolated from various vascular beds of the rat.
Methods. The aorta, middle cerebral artery, second-order mesenteric artery and first-order cremaster muscle artery were dissected from 6wk-old male Sprague-Dawley rats (sodium thiopentone 100 mg/kg i.p.). RAGE expression was determined using immunohistochemistry (IHC). Superoxide was assayed using the luminescent reagent L-012. Artery segments were incubated with unglycated or glycated BSA (glycating agents methylglyoxal (MGO) or glucose-6-phosphate (G6P) for 40 min at 37°C prior to the L-012 assay.
Results. RAGE expression was localised to the endothelium of the various artery types. RAGE expression was highest in the cremaster muscle artery, with lesser, similar levels of expression in the mesenteric and middle cerebral arteries, and a low level of expression in the aorta (n = 4 for each). Basal superoxide accumulation was highest in the middle cerebral artery, approximately double that observed in the cremaster muscle and mesenteric arteries with a very low amount of superoxide detected in the aorta (n = 6 for each). Superoxide accumulation was almost abolished by the NAD(P)H oxidase inhibitor apocynin (500 $\mu$M). Both MGO-BSA and G6P-BSA (0.1 mg/ml) inhibited superoxide accumulation in all vessels apart from the aorta; for example, MGO-BSA inhibited L-012 luminescence by 87 ± 8 % in the middle cerebral artery (n = 6, $P<0.05$). MGO-BSA (0.1 mg/ml) inhibited superoxide accumulation only when applied to the intra-luminal surface of the vessels.
Discussion. AGE inhibited superoxide accumulation in three of the vessels studied, presumably by inhibiting NAD(P)H oxidase. This effect did not appear to correlate with basal superoxide accumulation or RAGE expression.

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**Evidence for Reciprocal Dysregulation of Asymmetric Dimethylarginine and Myeloperoxidase in Atrial Fibrillation**

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Introduction: Atrial fibrillation (AF) has been associated with endothelial dysfunction and inflammatory activation, the latter mediated at least partially by myeloperoxidase (MPO). Previous investigations have suggested that MPO release is inhibited by nitric oxide (NO) and potentiated by the NOS inhibitor asymmetric dimethylarginine (ADMA). MPO also inhibits ADMA metabolism and increases NO catabolism in platelets, contributing to platelet activation. We have reported that the plasma protein thrombospondin-1 (TSP-1) inhibits NO signalling. To date these interactions have only been observed *in vitro*: the current study sought *in vivo* evidence of these interactions in AF.
Methods: Patients hospitalised with AF (n=106) were evaluated. Plasma MPO and TSP-1 concentrations were determined by ELISA, while ADMA was assayed by HPLC. Platelet reactivity to ADP and NO were determined via whole blood impedance aggregometry.
Results: There was a direct correlation between ADMA and MPO ($r=0.220$, $p<0.05$), and between MPO and TSP-1 ($r=0.221$, $p<0.05$). Platelet responsiveness to NO was not significantly correlated with MPO concentrations. Plasma TSP-1 concentrations were directly correlated with extent of ADP-induced aggregation ($r=0.254$, $p<0.01$).
Discussion: These data are consistent with the concept of a nexus between increased concentrations of ADMA (with resultant impairment of NO generation) and release of MPO (with resultant platelet activation and release of TSP-1). The combination of impaired NO effect and inflammatory activation may be critical to pathogenesis and outcomes in AF.

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**The DPP-4 inhibitor linagliptan improves endothelium-dependent relaxation of rat mesenteric arteries in the presence of high glucose and hyperglycaemia in STZ-induced diabetic rats.**

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Introduction. Dipeptidyl peptidase-4 (DPP-4) inhibitors are used to treat type 2 diabetes and may exert beneficial effects on the cardiovascular complications of diabetes, such as impairment of endothelial function, independently of their glucose lowering effects.
Aims. To investigate the effect of linagliptin, a DPP-4 inhibitor, on the mechanism(s) of endothelium-dependent relaxation in rat mesenteric arteries acutely exposed to high glucose or isolated from streptozotocin (STZ)-induced diabetic rats.
Methods. Endothelium-dependent and –independent relaxation to acetylcholine (ACh) and sodium nitroprusside (SNP) was determined in Wistar rat mesenteric arteries pre-contracted with phenylephrine (10-100 nM). Arteries were isolated from normal or diabetic rats and exposed to normal (11 mM) or high (40 mM) glucose.
Results. Endothelium-dependent relaxation was significantly impaired by exposure to high glucose or by hyperglycaimia in vivo (ACh $pEC_{50}$ 11 mM = 7.31±0.07, 40 mM = 6.32±0.21, diabetes = 6.15±0.21 $p<0.05$), but responses to SNP were not affected. Linagliptin (1μM) in vitro reversed the impairment of endothelium-dependent relaxation caused by high glucose and diabetes (ACh $pEC_{50}$ =7.25±0.06, 6.94±14 respectively). ACh-induced relaxation was also assessed when the contribution of NO was abolished by N-nitro-L-arginine (L-NNA, 100 μM) plus a soluble guanylate cyclase inhibitor (ODQ, 10 μM), or the contribution of endothelium derived hyperpolarising factor (EDHF) was inhibited with TRAM-34 (1μM) plus apamin (1μM). ACh-induced relaxation was significantly impaired by high glucose and diabetes under both conditions indicating that the contributions of both NO and EDHF were affected. Linagliptin significantly improved ACh-induced relaxation in the presence of both groups of inhibitors.
Discussion. Endothelium-dependent relaxation was impaired by high glucose and diabetes but was significantly improved by acute exposure to linagliptin which preserved the actions of both NO and EDHF demonstrating that the vasoprotective actions of the DPP-4 inhibitor are independent of any glucose lowering activity.

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**Impact of upregulated O-GlcNAcylation on left ventricular (LV) inotropic responsiveness in diabetic heart.**

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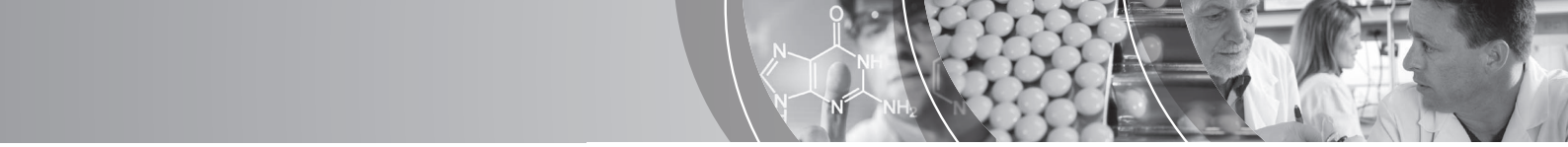
Introduction. Increased hexosamine biosythesis and downstream upregulated protein O-GlcNAcylation has been linked to diabetic complications in many tissues; its impact on LV contractile responsiveness is not well understood.
Aims. To determine the impact of acute and chronic upregulated O-GlcNAcylation on LV inotropic responsiveness.
Methods. Hearts isolated from anaesthetised adult male rats (ketamine:xylazine, 100:20mg/kg i.p.) were Langendorff-perfused (Krebs' at constant flow, 10ml/min). Baseline and phenylephrine-stimulated (PE, 10μmol/L) LV function was determined in response to acute (5μmol/L glucosamine, 30mins pretreatment) versus chronic upregulation of O-GlcNAcylation (8 weeks post-streptozotocin diabetes, 55mg/kg i.v.). Results were compared to untreated control or non-diabetic sham hearts, respectively.
Results. Chronic diabetes inhibited PE-induced inotropic responsiveness (Table, *$P<0.05$ vs control; $^{\#}P<0.05$ vs non-diabetic sham; peak at 6 mins); preliminary results also suggested a trend for diabetes-upregulated LV O-GlcNAc content (not shown). In contrast, acute O-GlcNAcylation with glucosamine did not fully reproduce the impaired PE inotropic response (Table), although baseline LV function was transiently reduced (not shown).

| PE-stimulated LV function (relative to baseline) | Control (n=6) | Acute ↑ O-GlcNAc (Glucosamine, n=7) | Non-diabetic sham (n=7) | Sustained ↑ O-GlcNAc (Diabetes, n=7) |
|---|---|---|---|---|
| LV Systolic Pressure (%) | 159 ± 22 | 165 ± 51 | 198 ± 24 | 124 ± 18$^{\#}$ |
| LV Developed Pressure (%) | 167 ± 17 | 159 ± 40 | 202 ± 31 | 114 ± 17$^{\#}$ |
| LV End-Diastolic Pressure (fold) | -1.4 ± 2.3 | -4.3 ± 5.3 | 0.9 ± 3.1 | 6.4 ± 3.8 |
| LV +dP/dt (%) | 162 ± 13 | 134 ± 27 | 175 ± 21 | 119 ± 19 (P=0.07) |
| LV –dP/dt (%) | 140 ± 5.6 | 96 ± 8.5* | 154 ± 25 | 108 ± 18 |
| Rate Pressure Product (%) | 161 ± 13 | 122 ± 16 (P=0.09) | 192 ± 34 | 132 ± 24 |
| Perfusion Pressure (%) | 125 ± 7.9 | 154 ± 26 | 132 ± 18 | 130 ± 16 |

Conclusion. These results support further assessment of the impact of upregulated protein O-GlcNAcylation on LV function, particularly in the diabetic heart.



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**Development of a UPLC-MS Based Approach to Quantify a Panel of Key Arginine Metabolites in Human Serum**

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Introduction: Cardiovascular disease (CVD) is the leading cause of mortality worldwide. Traditional risk factors (such as obesity, smoking and hypercholesterolaemia) predict approximately two thirds of CVD events (e.g. stroke, heart attack), leaving one third unexplained. Deficiency of the endogenous vasodilator nitric oxide (NO) is associated with important cardiovascular phenotypes including hypertension and hypercholesterolaemia. A key pathway in NO synthesis involves the conversion of arginine (ARG) to citrulline (CIT) by nitric oxide synthase (eNOS). However, ARG metabolism is complex, involving multiple interacting pathways that form various metabolites. In order to examine the role of ARG metabolism in NO synthesis, and thus its potential role in CVD, a comprehensive ARG metabolic profile must be considered.
Aims: Develop and validate an ultra-high performance liquid chromatography mass spectrometry (UPLC-MS) approach to simultaneously quantify key arginine metabolites in human serum.
Methods: ARG, asymmetric-dimethylarginine (ADMA), symmetric-dimethylarginine (SDMA), mono-methylarginine (L-MMA), homoarginine (hARG), CIT and ornithine (ORN) were extracted from serum (100µL) by the addition of 0.1% formic acid in methanol (500µL). The resulting supernatant (2µL) was analyzed by UPLC-MS. The serum concentration of each compound was quantified by comparison of peak area to calibration curves prepared using deuterated authentic standards.
Results: ARG, ADMA and ORN were detected as the base compounds at m/z 175.09, 203.15 and 133.14, respectively, while SDMA, L-MMA, hARG and CIT were detected as fragments at m/z 172.13, 116.09, 130.11 and 159.09, respectively. The method was validated with respect to intra- and inter-day variability (CV <15%), limit of detection and quantification (0.01x and 0.25x lower limit of reported reference ranges, respectively), and calibration curve linearity.
Discussion: A comprehensive UPLC-MS approach has been developed and validated to simultaneously quantify seven important compounds involved in arginine metabolism in human plasma. This approach facilitates correlation of an individual's arginine metabolic profile with cardiovascular phenotypes at population level.

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**Omega-3 fatty acids decrease ROS production in an apoE$^{-/-}$ mouse model of abdominal aortic dissection**

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Introduction: Reactive oxygen species (ROS) contribute to the pathogenesis of abdominal aortic dissection (AAD), produced by inflammatory and smooth muscle cells (Gavazzi et al 2007). Omega-3 polyunsaturated fatty acids have anti-oxidative effects (Brahmbhatt et al 2013), raising the possibility that they might protect against AAD.
Aim: To use an apolipoprotein E$^{-/-}$, angiotensin II-infused mouse model of AAD (apoE$^{-/-}$ angII), to investigate the effect of low omega-3 polyunsaturated fatty acid (LFA; 0.14%) and high omega-3 polyunsaturated fatty acid (HFA; 0.70%) diets on aortic ROS production and inflammatory cell infiltration.
Methods: ApoE$^{-/-}$ and C57 3-4 week old male mice were fed LFA or HFA diets for 8 weeks. Mice were infused with angII (1000 ng/kg/min; apoE$^{-/-}$ and C57) or 0.9% saline (C57), for 2 days. The abdominal aorta was processed for histology. Tissue levels of superoxide and peroxynitrite were detected and quantitated using dihydroethidium fluorescence and nitrotyrosine staining, respectively.
Results: Five angII C57 and angII ApoE$^{-/-}$ mice receiving LFA had a dissected abdominal aorta, compared to none receiving HFA ($P<0.05$). Neutrophil infiltration of the adventitia was greater in dissected (137±6.8/200 µm length, n=3), compared to non-dissected aortas (0.3±0.1/200 µm length, n=19; $P<0.05$). The amount of superoxide in adventitial inflammatory cells was lower in apoE$^{-/-}$ HFA mice (62.8±2.8 arbitrary units (AU), n=6) than apoE$^{-/-}$ LFA mice (90.6±3.8 AU, n=9; $P<0.05$). The amount of smooth muscle peroxynitrite was lower in C57 angII HFA mice (5.0±1.2 AU, n=9) than C57 angII LFA mice (14.2±3.4 AU, n=9; $P<0.05$).
Discussion: These findings suggest that a high omega-3 fatty acid diet is protective against aortic dissection. A possible mechanism is reduced ROS production by inflammatory and smooth muscle cells.

Brahmbhatt et al (2013) J Nutr Biochem 24:104-111
Gavazzi et al (2007) Hypertension 50:189-196

## 432

**Are $\alpha_1$-adrenoceptor antagonists effective in directly regulating smooth muscle tone in the human prostate gland?**

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Introduction. An increase in smooth muscle tone is a major component of Benign Prostatic Hyperplasia (BPH); arising in the transition zone (TZ), as opposed to the peripheral zone (PZ). However, the aetiology of BPH remains poorly understood, and the fundamental reason there is an increase in prostatic smooth muscle tone remains unknown. Our overall hypothesis is that changes in spontaneous activity significantly increase prostatic smooth muscle tone and contribute to the pathogenesis of BPH.

Aims. To test the effectiveness of $\alpha_1$-adrenoceptor antagonists to reduce the spontaneous activity of the human prostate gland.

Methods. TZ and PZ specimens were obtained from consenting patients undergoing radical prostatectomy. Subsequent recordings were made from prostatic preparations using conventional tension recording experiments (Dey A et al, 2010).

Results. The TZ had an increased resting basal tension of 3.70±0.54 mN, in comparison to 2.72±0.63 mN in the PZ. Spontaneous contractions in the TZ occurred at 1.81±0.30 $min^{-1}$, with amplitude of 0.21±0.03 mN, lasting 10.5±1.4 s. These were significantly less frequent (Student's paired t-test, $P<0.01$), smaller in amplitude (Student's paired t-test, $P<0.05$) and longer in duration (Student's paired t-test, $P<0.05$), in comparison to spontaneous contractions from 8 matched PZ specimens. Application of 1μM prazosin had no significant effects on spontaneous contractions in the TZ (Student's paired t-test, $P>0.05$, n=4). Application of 0.1nM tamsulosin significantly reduced the resting basal tension from 3.68±0.45 mN to 3.40±0.45 mN, and the amplitude from 0.27±0.06 N/g to 0.21±0.06 N/g in the TZ (Student's paired t-test, $P<0.05$, n=5).

Discussion. An increased resting basal tension in the TZ, in comparison to the PZ, demonstrates an increased smooth muscle tone in the region where BPH arises. Tamsulosin directly reduces but does not abolish the basal tension and amplitude of the spontaneous contractions in the TZ, which is likely to be associated with its clinical efficacy.

Dey A et al (2010) Br J Pharmacol 161(8):1692-1707

## 434

**Characterisation of relaxatory transmitters in the porcine internal anal sphincter**

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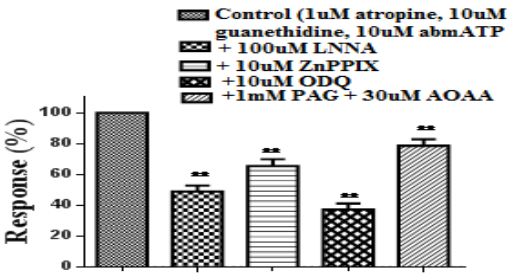



Introduction. The internal anal sphincter, contributes largely to maintenance of anorectal tone. Activity of the gastrointestinal tract smooth muscle is modulated by numerous endogenous neurotransmitters, and greater understanding of the relaxing neurotransmitters specific to the internal anal sphincter might reveal novel drug targets for faecal incontinence and anal fissures (Matsuda et al 2010a; 2010b).

Aims. To investigate the relaxatory neurotransmitters of the porcine internal anal sphincter.

Methods. Strips of pig internal anal sphincter were mounted in organ baths at 37°C in Krebs-bicarbonate solution. Strips were incubated for 30mins with atropine, guanethidine and αβmATP to block contraction induced by acetylcholine, noradrenaline and ATP, and subjected to electric field stimulation (EFS) (5Hz, 40V, and 1ms) in order to release endogenous neurotransmitters. Following the EFS, the strips were further incubated with NG-nitro-L-arginine, LNNA (nitric oxide synthase inhibitor), zinc protoporphyrin IX, ZnPPIX (hemeoxygenase inhibitor), 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one, ODQ (soluble guanylate cyclase inhibitor) or propargylglycine, PAG/aminooxyacetic acid, AOAA ($H_2S$ synthesis inhibitors) for another 30mins, and subjected to EFS.

Results. Relaxation of the internal anal sphincter is neurogenic as it was blocked by 1uM tetrodotoxin. In addition, inhibitors of NO, CO and $H_2S$ all reduced relaxation response of the internal anal sphincter to EFS (see Figure) (mean ± SEM, n=3-4; $p<0.05$); the tissue relaxation was not eliminated by combinatory addition of the inhibitors.

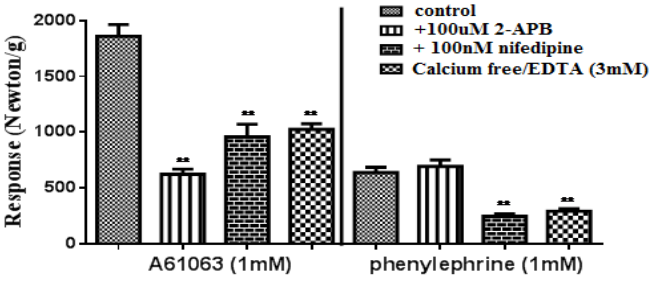
Discussion. These results shows that relaxation responses of the porcine internal anal sphincter involve endogenous CO, NO and $H_2S$. The remaining response is via an unknown neurotransmitter.

Matsuda N. et al (2010a). Fundam Clin Pharmacol, 24(3), 261-268.
Matsuda N. et al. (2010b). Acta Histochem, 112(4), 402-406.

435

**Characterisation of contractile responses to alpha1 adrenoceptor agonists in the porcine urethral circular smooth muscle**

Donna Sellers[1], Oladayo S Folasire[1], Russ Chess-Williams[1]. Faculty of Health Science & Medicine, Bond University[1], Gold Coast, Queensland, Australia.




Introduction. The circular smooth muscle, which makes up the urethral sphincter, is densely populated by $\alpha_{1A/L}$ adrenoceptors (Bagot, 2006). However, the intracellular signalling pathways underlying contractile responses to various $\alpha_1$ adrenoceptor agonists are not fully characterised in this tissue, and greater understanding may lead to novel targets for the treatment of stress urinary incontinence.
Aims. To investigate the calcium sources involved in contractile responses to phenylephrine and the highly potent $\alpha_{1A}$ agonist A61063 (Knepper et al, 1995) in the porcine urethra.
Methods. The circular smooth muscle strips were mounted in organ baths at 37°C in Krebs-bicarbonate solution. Contractile responses to the $\alpha_1$ adrenoceptor agonists A61063 and phenylephrine were obtained in the absence and presence of calcium channels inhibitors (nifedipine and 2-aminethoxydiphenyl borate, 2-APB), and in calcium-free Krebs solution.
Results. Maximum response to A61063 was significantly greater than that of phenylephrine. Responses to both agonist were reduced by nifedipine and extracellular calcium exclusion (see Figure), while 2-APB also reduced activation to A61063 (but not in phenylephrine) (mean ± SEM, n= 3-7; $p<0.05$).
Discussion. Responses to both agonists involve extracellular calcium influx while responses to A61063 also involve additional intracellular calcium release.

Bagot K. et al(2006). Autonomic and Autacoid Pharmacology, 26:345-353.
Knepper S. M. et al(1995). J Pharmacol Exp Ther, 274:97-103.

436

**Bile acids induce itch via a TGR5-TRPA1 dependent pathway**

Gihan Jayaweera[1], TinaMarie Lieu[1] & Nigel W. Bunnett[1]. Monash Institute of Pharmaceutical Sciences, Parkville, Victoria, Australia

Introduction. Cholestasis is characterised by impaired biliary flow in the liver which leads to the elevation of bile acids in the circulation. A proportion of these patients experience a profound itch by an unknown mechanism. In mice, TGR5 (Gs coupled GPCR) has been shown to mediate bile acid induced itch via a histamine independent pathway (Alemi et al, 2013). TRPA1 (ion channel) has been shown to couple to GPCRs to induce histamine independent itch. We hypothesise that TGR5 couples to TRPA1 to cause bile acid induced itch.
Aims. To determine whether bile acids induce itch via a TGR5-TRPA1 dependent pathway.
Methods. We analysed scratching behaviour in wild-type and TGR5 overexpressing (TGR5-tg) mice. We treated the animals with bile acids, bile acid sequestrants and TRPA1 antagonists. Intracellular cAMP was measured in HEK293 and HEK-TGR5 cell lines following treatment with plasma from healthy patients and patients with cholestasis (+/- itch).
Results. Colestipol (bile acid sequestrant) reduces the spontaneous itch seen in TGR5-tg mice (n=8). Administration of a TRPA1 antagonist (HC-003031, 100mg/kg, i.p.) reduced both spontaneous itch in TGR5-tg mice (n=8) and bile acid induced itch in wild-type mice (n=6). Plasma from patients with cholestatic itch activates HEK293-TGR5 cells but not HEK293 cells (n=6). Plasma from healthy controls and patients with cholestasis without itch did not activate HEK-TGR5 cells.
Discussion. The ability of colestipol to decrease spontaneous itch seen in TGR5-tg mice suggests that overexpressing TGR5 may amplify the effect of endogenous circulating bile acids to induce itch. Antagonising TRPA1 abolishes the itch response indicating that TRPA1 is required for transmission of itch. Plasma from patients with cholestatic itch activates HEK cells via a TGR5 dependent mechanism, which implies that a component of the plasma in these patients induce itch by activating TGR5. Therefore, bile acids induce itch by activating TGR5 via TRPA1.

Alemi F et al (2013). The Journal of Clinical Investigation. 123(4):1513-30

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**The effect of age on contractile responses of the porcine ureter to carbachol and phenylephrine**

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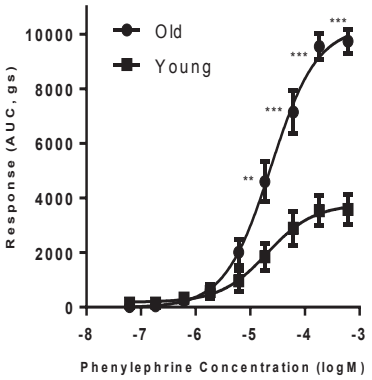


Introduction. Aging has been reported to increase the risk of ureteral calculus development (Costa-Bauza et al, 2007). This condition is frequently accompanied with ureteral colic which is understood to be caused by constriction of the ureteric tube, initiated by smooth muscle contractions. The cholinergic and α-adrenergic systems have been shown to increase ureteric contractions and hence, have significant roles in ureteral obstruction (Canda et al, 2007).

Aim. To compare the effects of phenylephrine and carbachol on isolated ureters from old and young pigs.

Methods. Contractile responses of isolated circular smooth muscle strips to carbachol and phenylephrine were examined in distal ureteral tissues from young (20 weeks) and old (56 weeks) pigs. Tissues developed spontaneous contractile activity and responses were expressed as area under the curve (gs) normalized to tissues weights.

Results. The potency ($pEC_{50}$) of phenylephrine was similar in tissues from young and old animals (4.69±0.21 vs 4.62±0.08). However, maximal contractions to phenylephrine were greater (P<0.0001) in tissues from older animals than those from young animals (Figure 1, both groups n=12). Tissues from young animals failed to respond to carbachol (n=12), but in older animals, ureteral strips developed contractile activity (maximum response = 5201.71±887.17gs, n=12)

Discussion. These results suggest that tissues from older animals have greater responses to both muscarinic and α-adrenergic receptor stimulation which may increase the likelihood of ureteral obstruction.

Canda et al (2007) Urol Int 78:289-298.
Costa-Bauza et al (2007) World J Urol 25:415-421.

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**Effects of temperature and incubation times on cellular localization of $\alpha_{1A}$-adrenoceptors labeled with BODIPY FL-prazosin (QAPB)**

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Drug Disc Biol, Monash Inst of Pharmaceutical Sci[1], Monash Univ, Parkville, VIC.

Introduction. $\alpha_{1L}$-adrenoceptors are a functional phenotype of the $\alpha_{1A}$-adrenoceptor that is responsible for smooth muscle contractions of the prostate as well as other tissues in the lower urinary tract. Previous studies involving radioligand binding in whole cells exhibited $\alpha_{1L}$-adrenoceptor pharmacology whereas homogenized prostatic membrane preparations showed $\alpha_{1A}$-adrenoceptor pharmacology (Nishimune *et al*., 2010). This suggests that the integrity of the cell membrane is important to observe the $\alpha_{1L}$-adrenoceptor phenotype. In this study we hypothesize that cellular localization of the receptor is important to the interpretation of these pharmacological findings.

Aims. To optimize a fluorescent based imaging assay to determine the cellular distribution of $\alpha_{1A}$-adrenoceptors and their binding characteristics in live CHO cells expressing the human $\alpha_{1A}$-adrenoceptor using the fluorescent ligand, BODIPY-FL prazosin (QAPB).

Methods. Live cells seeded at 40000cells/well labeled with 10nM QAPB for 5 minutes and 1hour was imaged in 96 well plates in the presence and absence of $\alpha_1$-adrenoceptor antagonists (1pM - 10$\mu$M) at 25° and 37°C using IN Cell Analyzer 2000. Binding affinities were then determined for each antagonist under the different conditions.

Results. QAPB labelled mainly cell surface receptors at 25°C when exposed for 5 minutes whereas at 37°C, a mixture of cell surface and intracellular receptors were labelled. When QAPB was incubated for 1 hour, an increase in intracellular receptors was observed for both 25°C and 37°C.

Discussion. Only $\alpha_{1A}$-adrenoceptor and not $\alpha_{1L}$-adrenoceptor pharmacology was observed in CHO cells despite the existence of intracellular and membrane bound populations of receptors.

Nishimune A, Suzuki F, Yoshiki H, Morishima S, Muramatsu I (2010). Alpha 1-adrenoceptor pharmacome: alpha 1L-adrenoceptor and alpha 1A-adrenoceptor in the lower urinary tract. *International journal of urology : official journal of the Japanese Urological Association* **17**(1)**:** 31-37.

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**The role of liver sinusoidal endothelial cells in the pathogenesis of insulin resistance**

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Introduction. The liver plays a major role in the regulation of glucose homeostasis, which was tightly regulated by insulin. Prior to hepatic metabolism, substrates travel through fenestrations in the liver sinusoidal endothelial cells to gain access to hepatocytes. We propose loss of fenestrations (defenestration) impair the transfer of insulin and glucose across the hepatic sinusoidal endothelium, thus contributing to hepatic insulin resistance.
Aims. To investigate the effect of defenestration on glucose homeostasis in rats treated with poloxamer 407 (P407), a synthetic surfactant that causes defenestration and hyperlipidemia.
Methods. Radiolabelled glucose tolerance test was performed in control rats (n=10) and rats injected intraperitoneally with P407, 24h prior to experimentation (1g/kg, n=10). Rats were fasted for 8h and injected with glucose (2g/kg i.p.) spiked with 10 $\mu$Ci $^{14}$C-glucose for assessment of insulin action and 10 $\mu$Ci $^{3}$H-2-deoxyglucose for assessment of glucose uptake. Blood glucose level were read at 0, 15, 30, 45, 60 and 90m using glucometer and insulin level were determined at the beginning and end of experiment for calculation of Homeostatic Model Assesment (HOMA) index. Liver, white adipose tissue (WAT) and muscle were snap frozen for radioactivity analysis at the end of experiment.
Results. Loss of fenestrations in the P407 treated rats induced hyperinsulinemia (T0: Control 0.61±0.07 vs P407 1.35±0.22, p=0.005; T90: Control 0.94±0.15 vs P407 2.85±0.73, p=0.036) and reduced insulin sensitivity (HOMA index; Control 5.43±0.68 vs P407 11.7±2.06, p=0.014). In addition, there was decreased incorporation of glucose into glycogen in the liver (Control 4.2±0.38 vs P407 2.8±0.48, p=0.042), but not in muscle and WAT in the P407 treated group. P407 did not affect in glucose uptake in muscle and WAT.
Discussion: The defenestrated model is insulin resistant, with impaired glucose homeostasis particularly in the liver. It further affirms the important role of the liver ultrastructure in hepatic metabolic processes and highlighting it as an important potential therapeutic target.

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**Loss of Vitamin C synthesis worsens liver defenestration of the Wrn$^{\Delta hel/\Delta hel}$ mouse model-preliminary analysis.**

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Introduction. The liver is central to the ageing process because it plays a pivotal role in many metabolic and detoxification processes that impact on ageing and disease susceptibility. The Werner syndrome (Wrn$^{\Delta hel/\Delta hel}$) transgenic mouse model is of great interest for studying ageing changes in the liver because it develops atherosclerosis and hypertriglyceridaemia, conditions associated with age-related changes to the endothelium of the liver blood vessels (sinusoids), known as pseudocapillarization. These changes include loss of the endothelial holes, known as fenestrations and deposition of extracellular matrix. We have previously shown that some of these changes can be reduced through vitamin C supplementation in the Wrn$^{\Delta hel/\Delta hel}$ mice. Here we further investigate the role of Vitamin C in the integrity of the liver sinusoidal endothelium of Wrn$^{\Delta hel/\Delta hel}$ by crossbreeding Wrn$^{\Delta hel/\Delta hel}$ and Gulo transgenic mice, which are unable to synthesize Vitamin C.
Aims. To investigate the liver sinusoidal endothelium in the double transgenic mouse model Wrn$^{\Delta hel/\Delta hel}$/Gulo..
Methods. Livers of Wrn$^{\Delta hel/\Delta hel}$/Gulo transgenic Gulo transgenic, Wrn$^{\Delta hel/\Delta hel}$ and wild type control mice aged 5 and 18 weeks were needle perfused to preserve morphology for electron microscopy. Samples were then analysed using scanning electron microscopy using standard techniques.
Results. Preliminary results show that the Wrn$^{\Delta hel/\Delta hel}$ mice undergo sinusoidal endothelial changes by 18 weeks and that the double Gulo/Wrn$^{\Delta hel/\Delta hel}$ mutation induces further significant changes in the sinusoidal endothelium, including significant loss of fenestrations. The Gulo mutation alone does not induce sinusoidal endothelial changes by 18 weeks.
Discussion. This study suggest Vitamin C has significant implications for the integrity of the liver sinusoidal endothelium in the Wrn$^{\Delta hel/\Delta hel}$ model of the ageing liver and further analysis will contribute to our understanding of age-related changes in liver function.

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**$\alpha_{1a}$-adrenoeptors stimulate glucose uptake via mTORC2, AMPK, and Rac1**

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Introduction. The metabolic changes that occur in cancer cells involve an increase in glucose uptake and utilisation (Hamanaka et al, 2012). Prostate cancer cells have high $\alpha_{1a}$-adrenoceptor ($\alpha_{1a}$-AR) expression and reprogrammed cellular pathways controlling proliferation, survival and metabolism (Anglin et al, 2002).
Aims. Signalling pathways involved in $\alpha_{1a}$-AR mediated glucose uptake were investigated in CHO-K1 cells and 22RV1 prostate cancer cells that may lead to the identification of a selective mechanism for killing cancer cells.
Methods. Signalling pathways mediating glucose uptake were investigated using selective kinase inhibitors or siRNA and detected by measuring [$H^3$]-glucose uptake, western blots, α-screen assays and a cell proliferation assay.
Results. Noradrenaline (NA), and the selective $\alpha_{1a}$-AR agonists A61603 and oxymetazoline dose-dependently increased glucose uptake in CHO-K1 cells stably expressing the $\alpha_{1a}$-AR. AMPK inhibitor and siRNA of mTORC2 component rictor significantly inhibited NA, A61603, and oxymetazoline-mediated glucose uptake. Rac1 inhibitor NSC23766 inhibited glucose uptake stimulated by NA, A61603, and oxymetazoline by 85%, 80%, and 71% respectively. In 22RV1 cells, NA and A61603 dose-dependently increased glucose uptake that was significantly inhibited by NSC23766 and mTOR inhibitor KU0063794. Western blot and α-screen assay showed $\alpha_{1a}$-AR agonists have no effect on Akt and Erk phosphorylation but increased phosphorylation on mTOR at Ser2481 and AMPK.
Discussion. $\alpha_{1a}$-AR mediate two separated pathways which activate mTORC2 and AMPK, and Rac1 has a key role for glucose uptake potentially due to actin reorganization which translocates glucose transporters to the plasma membrane.

Anglin IE et al (2002) Prostate Cancer Prostatic Dis 5(2):88-95
Hamanaka RB et al (2012) J Exp Med 209(2):211-5

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**Characterisation of heteromeric 5-$HT_3$ receptors: focusing on the 5-$HT_{3C}$ and 5-$HT_{3E}$ subunits**

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Introduction. 5-$HT_3$ receptors are ligand-gated cation channels present in both central and peripheral nervous systems. 5-$HT_3$ receptor antagonists are used clinically for a range of disorders including irritable bowel syndrome and chemotherapy-induced nausea but have varying efficacy. 5-$HT_3$ receptors potentially contain five different subunits (A, B, C, D and E) that may contribute to the variability in efficacy (Yaakob et al. 2011).
Aims. To characterise expression and function of 5-$HT_3$ receptors containing C or E subunits.
Methods. Quantitative real-time PCR of human colonic tissue was used to determine mRNA expression for all 5-$HT_3$ receptor subunits. Whole cell patch clamping was used to functionally characterise recombinant 5-HT receptors expressed in HEK293T cells.
Results. Expression patterns of the A, B and C subunits did not vary between human colonic tissues or regions. However the E subunit was found predominantly in mucosa but not muscle layers. Heteromeric receptors containing the A and C or E subunits exhibited different pharmacological profiles to 5-HT and the antagonists, ondansetron and palonsetron.
Discussion. The widespread distribution of the C and E subunits throughout the human colon suggest that they may contribute to physiological functions. The predominant location of the E subunit in the mucosa could make it a potential therapeutic target. Patch-clamp experiments indicate that C and/or E subunits in hetomeric receptors alter efficacies of clinically used antagonists ondansetron and palonosetron, which could contribute to inadequate response observed in 20-40% proportion of patients.

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

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**Development and evaluation of microemulsion formulations for transdermal delivery of caffeine**

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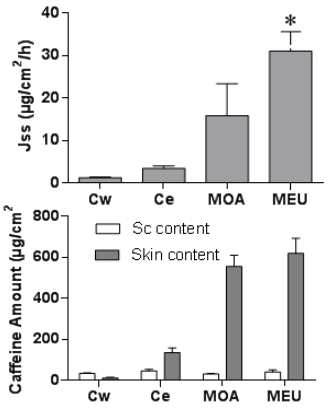
Introduction Microemulsions are increasingly used as efficient vehicles for topical drug delivery. The penetration of hydrophilic drugs, which do not readily cross the skin barrier, may be enhanced by such formulations.

Aim To evaluate microemulsion formulations containing oleic acid (OA) and eucalyptol (EU) as oil phases for improved skin delivery of hydrophilic drugs, using caffeine as a model compound.

Methods Two oil-in-water (O/W) microemulsion formulations containing caffeine (3%) and either OA or EU as oil phases were prepared, with oil, surfactant, co-surfactant and aqueous phase proportions chosen with pseudo ternary phase diagrams. Particle size (by DLS), conductivity, viscosity, refractive index and pH were evaluated. Microemulsions (MOA & MEU) and controls (3% caffeine in water (Cw) or 60% ethanol/water (Ce)) were applied to full thickness excised human abdominal skin in Franz diffusion cells (n=4) for 8 h and caffeine concentrations in receptor fluid and extracts of tape strips (20) and skin were measured by HPLC.




Results Caffeine flux and amount extracted from stratum corneum (tape strips) residual skin were all significantly greater for the microemulsions than controls. Caffeine flux from the MEU was significantly greater than MOA (31.0 ± 4.6 vs 15.8 ± 7.5 $\mu$g/cm$^2$/h; P<0.005) but SC and skin content were not significantly different for these formulations.

Discussion In conclusion, both the microemulsion systems in this study gave significantly enhanced penetration of a model hydrophilic drug, caffeine. Further developments will allow better targeting to specific skin layers. The enhancement may be partly due to alterations in the stratum corneum barrier structure by eucalyptol and oleic acid, which are known penetration enhancers.

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**Over-the-counter interventions and advice for acute low back pain: systematic review and meta-analysis**

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Introduction: Low back pain is a condition for which over-the-counter (OTC) interventions and advice are readily sought, however there is uncertainty around the effectiveness of OTC interventions that can be delivered for acute low back pain (ALBP).

Aims. To evaluate evidence for the clinical benefits of OTC interventions and advice that could be provided to people with ALBP.

Methods. Searches were conducted on MEDLINE, EMBASE, Cochrane Database of Systematic Reviews, AMED, CENTRAL and PsychINFO for eligible RCTs. Two reviewers extracted data and rated study quality. The primary outcome measure was pain. Eligible controls included placebo, no treatment or usual care. A random effects model was used to pool trial effects with the overall strength of evidence described using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria.

Results. Thirteen RCTs (2847 participants) evaluating advice, bed rest, simple analgesics (paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs)), heat application and a topical rubefacient were included. There is low quality evidence that bed rest is ineffective and very low quality evidence that advice is ineffective in the short, intermediate and long term. There was very low quality evidence that NSAIDs (ibuprofen and diclofenac 'when required' dosing) provide an analgesic effect in the immediate term Mean Difference (MD) -10.9 [95% CI -17.6, -4.2] and -11.3 [95% CI -17.8, -4.9] respectively. There is very low quality evidence that heat wrap and a capsicum-based rubefacient provide an analgesic effect in the immediate term MD -13.5 [95%CI -21.3, -5.7] and 17.5, p<0.001 respectively but there was no information on longer term outcomes.

Discussion. There is limited evidence that ibuprofen, diclofenac, heat wrap and a capsicum-based rubefacient provide pain relief for ALBP and that bed rest and advice are ineffective. Future research is needed to support rational use of OTC remedies and advice for people with ALBP.

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**Estimating fat-free mass in children**

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Introduction. Fat-free mass (FFM) is an important covariate for estimating the clearance of drugs. Mathematical models for estimating FFM have been developed in adults but there are currently no models available to predict FFM in children.
Aims. The aim of this project was to develop and evaluate a model to predict FFM in children.
Methods. Two types of models (M1, M2) were developed to describe FFM in children. M1 was an empirical model that contained all possible statistically significant covariates and interactions and was developed in STATA v11. M2 was a simpler sigmoid hyperbolic model and was developed in NONMEM v7.2. The models were built from an index dataset (496 females and 515 males). M1 was developed to provide the best possible description of the data (i.e. a positive control). In addition, a published adult model (M3) was applied directly as a naive description of the data (i.e. a negative control). The predictive performances of the three models were assessed using mean error (ME) and root mean squared error (RMSE). A test dataset (90 females and 86 males) was available for external evaluation.
Results. M1 consisted of 9 terms with up to 2$^{nd}$ level interactions (age, sex, height, weight, tanner score, and bone mass). M2 was a sigmoid hyperbolic model based on age with an asymptote at the adult prediction (M3). For the index data set, the ME for M1, M2 and, M3 were 0.17 (95% CI 0.03 – 0.30), 0.26 (0.11 – 0.42), and 0.29 (0.06 – 0.51), respectively and RMSE were 2.18 (2.02 – 2.35), 2.45 (2.28 – 2.64), and 3.76 (3.54 – 3.97) kg. For the test data set, the ME for M1, M2 and, M3 were -0.18 (-0.46 – 0.13), -1.39 (-1.90 to -0.92), and -1.49 (-2.10 to -0.91) kg, respectively and RMSE were 2.08 (1.73 – 2.44), 3.60 (2.89 – 4.45), and 4.33 (3.52 – 5.19) kg.
Discussion. A general maturation model for FFM in children was developed. This was compared to an empirical model in a large dataset and was found to perform well. The model was externally evaluated by predicting into a test dataset and was shown to have good predictive performance compared to the published adult model.

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**The inflammation-resolving trihydroxydocosahexaenoic acid derivative, resolvin D2, supports MCF-7 cell proliferation via activation of estrogen receptor**

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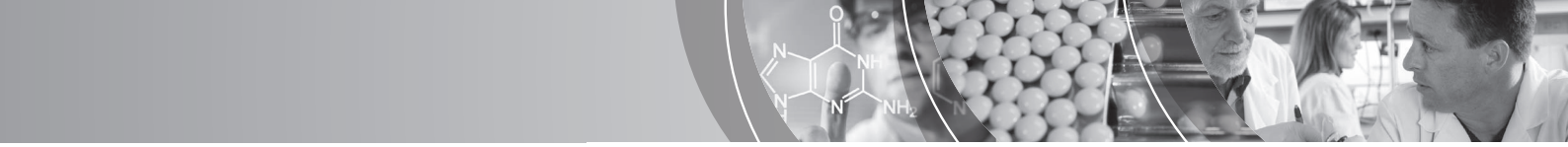
Introduction: Resolvin D2(RvD2) is an inflammation-resolving tri-hydroxy lipid mediator generated endogenously from the omega-3 polyunsaturated fatty acid, docosahexaenoic acid (DHA) (Serhan et al., 2011). Formyl peptide receptors (FPRs) are G-protein coupled receptors expressed in both estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) breast cancer cell lines. Endogenous inflammation-resolving FPR ligands (annexin A1 and its N-terminal peptide Ac2-26, and the tri-hydroxy lipid mediator lipoxin $A_4$) stimulate the proliferation of both MCF-7 and MDA-MB-231 cells through activation of FPR (Khau et al, 2011).
Aim: To investigate the impact of RvD2 on breast tumour cell proliferation.
Methods: Resolvin D2 was obtained by total chemical synthesis. Both MCF-7 and MDA-MB-231 were seeded in 10% FCS media for 24 hours. Cells were then incubated with serum free media for another 24 hours before treatment with FCS 5% (v/v) or RvD2 0.1-100 nM. After 48 hours, viable cells were enumerated. MCF-7 cells were transfected with estrogen response element (ERE)-controlled secretory alkaline phosphatase (SEAP) and pGL3 luciferase vector. MCF-7 cytosol was incubated with increasing concentrations of estradiol or RvD2 in the presence of $^{3}$[H]E2.
Results: RvD2 was mitogenic for MCF-7 ($P<0.0001$), but not MDA-MB-231 cells. (100nM) RvD2 mitogenesis was attenuated by FPR antagonists and prevented by estrogen receptor antagonist, ICI 182,780 (100nM) ($P<0.0001$). RvD2 stimulated ERE activity but did not affect $^{3}$[H]E2 binding to MCF-7 cytosol.
Conclusion: RvD2 induces the proliferation of MCF-7, but not MDA-MB-231 cells, through actions dependent on estrogen receptor but not involving binding to the estrogen orthosteric site.

Khau T et al (2011) FASEB J 25, 483-496.
Serhan N et al (2011) Curr Top Med Chem 11, 629-647.



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**Dehydration and diuretic use are common in older patients presenting with falls, particularly amongst the frail**

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Introduction. In older people, falls are caused by the interaction of multiple predisposing risk factors. Medication effects are common potentially preventable or reversible causes of falls. Dehydration also contributes to the risk of falls, and may be precipitated by diuretic use. The coexistence of dehydration and diuretics in robust and frail hospitalised fallers has not been documented.

Aims. To measure the prevalence of dehydration and diuretic use in robust and frail older patients presenting with falls.

Methods. Patients ≥60 years admitted with a fall were recruited from a Sydney teaching hospital. Demographic, clinical, medication and falls data were collected at admission. Dehydration was defined as serum sodium concentration >145 mEq/L or serum urea:creatinine ratio >100. Diuretic exposure was defined as prescription of loop, thiazide or potassium sparing diuretics immediately prior to admission. The Reported Edmonton Frailty Scale assessed frailty.

Results. 204 (103 frail, 101 robust) participants were recruited with a mean age of 81 (± 8.3) years; 65% were female. Compared to robust, frail older fallers were more likely to use diuretics (34% frail, 14% robust; p=0.001), to be dehydrated (20%, 7%; p=0.001) and to have both diuretic use and dehydration (13%, 3%; p=0.01). In those who were dehydrated, diuretic use was significantly more common in the total (diuretic users 33%, non-diuretic users 7%; p<0.0001), frail (37%, 10%; p=0.001) and robust (21%, 5%; p=0.02) participants.

Discussion. Dehydration, potentially exacerbated by diuretic use, is common amongst older patients, particularly the frail, admitted after a fall. Diuretic use is a potentially reversible iatrogenic risk factor for falls in older people and should be reviewed frequently, especially in the frail.

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**Prevalence of chemotherapy dose reductions in obese women with breast cancer**

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Introduction. Obese women with breast cancer have 30% worse survival than non-obese women. We postulated that this was due to relative under-dosing for body size in obese compared to non-obese women.

Aims. To compare body size-adjusted chemotherapy dose between obese and non-obese women undergoing adjuvant treatment of breast cancer.

Methods. We conducted a retrospective audit of 550 women treated since 2000 with adjuvant chemotherapy for breast cancer at a large tertiary hospital in Brisbane, Australia. Cases were identified from the hospital's chemotherapy database. Subject, tumour and chemotherapy data was extracted from patient charts. Dosing was analysed by comparing expected dose based on patient body surface area to actual dose received. A multivariate analysis was performed examining dose reductions across patient and tumour characteristics.

Results. 358 women had complete data available and were eligible for inclusion. In this population 30.4% (n=109) were obese with a body mass index greater than 30kg/m$^2$. An initial dose reduction was independently associated with obesity (OR=7.27; 95% CI 1.35 to 39.15; $p$=0.021), and diabetes (OR=8.16; 95% CI 1.13 to 58.78; $p$=0.037). Overall in the first cycle, obese women were dosed significantly less for their body size with a median 98.0% of expected dose based on actual body size, compared to 99.8% in non-obese women ($p$<0.001).

Discussion. Obese women now account for a large proportion of breast cancer patients. These women are relatively under-dosed for body size compared to non-obese woman. The results confirm altered treatment of obese women with doses being reduced from the initiation of treatment. This may be a contributing factor to the survival disadvantage observed in obese women, compared to non-obese women with breast cancer. The results in this study can contribute to the development of Australian guidelines in chemotherapy dosing for obese women with breast cancer.

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**Understanding and improving aminoglycoside use**

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Introduction. Aminoglycosides can effectively treat Gram-negative infections, however, inappropriate dosing regimens can result in significant nephrotoxicity and ototoxicity. The *Australian Therapeutic Guidelines: Antibiotic* were revised in 2010 and aimed to individualise dosing based on renal function, age and weight. Since guideline revision, two studies have examined use of aminoglycosides in hospitals, but the hospitals did not have access to electronic prescribing and reasons for non-compliance were not explored.
Aims. To determine whether aminoglycoside prescribing at St Vincent's Hospital is compliant with the most recent guidelines and to explore the barriers that limit optimal use.
Methods. Phase 1: A retrospective audit of gentamicin, tobramycin and amikacin at St Vincent's Public Hospital in 2012. Data were extracted from various electronic record databases. The main outcome measures were adherence to the guidelines in terms of doses prescribed, dosing intervals and monitoring. Phase 2: Prescribers from medical units with high volume aminoglycoside prescribing were identified and interviewed about their use of aminoglycosides.
Results. Aminoglycosides were prescribed 1026 times for 654 patient cases. Dosing intervals in short-term intravenous therapy were appropriate in 71% of cases. Monitoring was performed in only 33 of the 60 prolonged therapy cases (>72hrs). Doctors reported prescribing aminoglycosides either out of habit or as part of a protocol. Their fear of potential toxicity resulted in doses that were often conservative. Doctor's awareness of appropriate monitoring and other aminoglycoside support services available at the hospital was poor.
Discussion. Prescribing of aminoglycosides at this hospital was found to be sub-optimal with respect to the therapeutic guidelines. Even with the hospital's electronic prescribing system, doctor awareness of critical issues in aminoglycoside therapy such as, monitoring frequency and timing in prolonged therapy and of the computerised dose prediction service was poor. A need was identified to make information more accessible within the electronic prescribing system.

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**The management of medicines in general practice by subjects with diabetes and high glycosylated haemoglobin levels**

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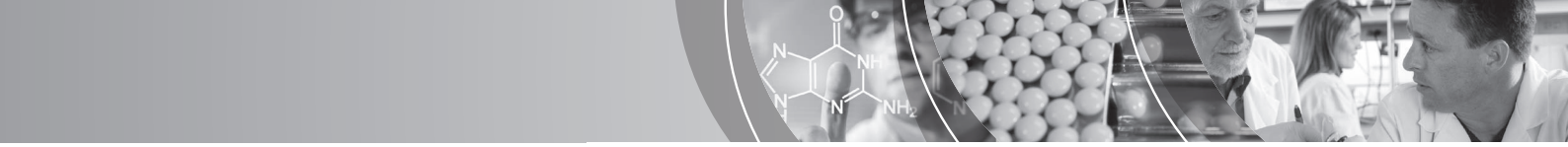
Introduction. Poor adherence to medicines, in subjects with diabetes, increases the risk of cardiovascular disease, kidney failure and blindness. Most studies have shown an association between adherence to anti-diabetic medicines and HbA1c levels. Thus, subjects with high HbA1c levels are often considered to have low adherence to medicines.
Aims. The aim of this study was to explore the management of medicines, including adherence, in subjects with poorly managed diabetes, as indicated by HbA1c values ≥ 7.2% in a large general practice in a low socioeconomic area.
Methods. The method was a semi-structured interview focussing on (i) diabetes and demographics, (ii) medicines being taken, and (iii) adherence to medication. Adherence to medicines was measured on the Morisky scale, which measures current adherence, and by the Doggrell-Kairuz perception method, which considers present and future adherence.
Results. Ten subjects (8 males) with poorly controlled diabetes (mean HbA1c was 8.8%) participated, and were being managed by 4 different GPs. On the Morisky scale, 70% were highly adherent and 30% had an intermediate level adherence. With the Doggrell-Kairuz method, 60% were fully adherent and likely to remain so, 20% were partially adherent and for these, it was difficult to predict what was going to happen in future. The remaining 20% were poorly adherent and this was likely to be ongoing. Despite high HbA1c levels, 6 of the subjects were only taking one medicine to manage their diabetes, and 4 of these were highly adherent. Two of the three subjects taking two medicines for diabetes were also highly/fully adherent.
Discussion. This study shows that most of the subjects with high HbA1c levels in general practice have good adherence to medicines, and the reason for their high HbA1c levels may be that they are under-medicated.

Doggrell SA, Kairuz T (2012) J Pharm Pract Res 42(3); 208-212.56:23-33



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**Rethinking medicines decision-making in Australian hospitals: Guiding principles for the quality use of off-label medicines**

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Introduction: Off-label use of medicines is common in Australian hospitals. Associated clinical, safety, ethical, legal and financial issues require a systematic approach to ensure delivery of Quality Use of Medicines (QUM).
Aims: To develop a consensus framework for the quality use of off-label medicines in Australian public hospitals.
Methods: A literature review was undertaken to define "off-label medicines use" terminology, describe its extent, and associated clinical, ethical/legal, and governance issues. A draft set of Guiding Principles was developed by the CATAG project team. An Expert Advisory Group (EAG) was convened, comprising expertise in therapeutics/QUM; evidence-based medicine; clinical medicine and clinical pharmacy (adult and paediatric); nursing and consumer issues. Clinicians from key therapeutic areas with demonstrated high use of off-label medicines were included. The EAG met face-to-face in May 2013 to review and refine the proposed draft principles and accompanying decision-algorithm. The revised draft was circulated to CATAG members and relevant external organisations for comment in August 2013. All feedback received will be collated, reviewed and discussed to refine the final content of the Guiding Principles and make recommendations for future work.
Results: Six overarching Guiding Principles provide key recommendations for the quality use of off-label medicines: 1. Use high quality evidence to determine appropriateness of off-label medicines use; 2. Involve the patient/carer in shared decision-making when recommending the use of an off-label medicine; 3. Consider review by the Drug and Therapeutics Committee when prescribing an off-label medicine; 4. Ensure appropriate information is available at all steps of the medicines management cycle; 5. Monitor outcomes, effectiveness and adverse events; 6. Consider liability and accountability when using medicines off-label. A decision-algorithm provides additional guidance.
Discussion: These Guiding Principles will assist and standardise decision-making by health care professionals, Drug and Therapeutics committees and consumers in their evaluation, approval and use of off-label medicines.

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**The effect of old age and frailty on intravenous fentanyl and midazolam pharmacodynamics**

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Introduction. Extensive variability in physiology, PK and PD is observed in older persons aged 65+ years, and may be partially explained by frailty. Plasma concentrations of interleukin (IL)-6, C-reactive protein (CRP) and albumin may be useful biomarkers of frailty in conjunction with clinical measures. The CL of iv fentanyl and midazolam is reduced in old age. The impact of frailty on PD sensitivity to the sedative and chronotropic effects of these drugs is unknown.
Aims. (1) Evaluate the use of IL-6, CRP and albumin as biomarkers of frailty; (2) Compare changes in sedation and heart rate between young non-frail, old non-frail and old frail patients receiving iv fentanyl and midazolam for sedation during endoscopy.
Methods. Patients aged over 45 years were recruited from the endoscopy unit at Royal North Shore Hospital, Sydney. Baseline blood was used to measure CRP and albumin (by hospital pathology) and IL-6 (using ELISA). Frailty was assessed using the Reported Edmonton Frail Scale (REFS). Heart rate and Ramsay sedation score were measured at baseline and at regular intervals for 2 h after drug administration.
Results. Plasma CRP, IL-6 and albumin concentrations were not significantly different between the groups. CRP positively correlated with REFS score ($P<0.05$) but not age. IL-6 and albumin did not correlate with age or REFS score. Old frail patients received lower doses of fentanyl and midazolam compared to old non-frail patients ($P<0.05$). No change in heart rate was observed between or within any group over 2 h. Maximum change in sedation score was significantly predicted by total weight-adjusted midazolam dose ($P<0.05$) but not old age or frailty.
Discussion. In this cohort, CRP, IL-6 and albumin were not useful as biomarkers of frailty. Old age and frailty were not associated with altered sensitivity to the sedative and chronotropic effects of fentanyl and midazolam.

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**Understanding and improving allopurinol use in a tertiary hospital**

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Introduction. Allopurinol inhibits uric acid synthesis and is widely used for gout prophylaxis. Maintenance doses of allopurinol have conventionally been based on patients' creatinine clearances to decrease risk of allopurinol hypersensitivity. New guidelines advocate the use of higher allopurinol doses and titrating doses against serum uric acid (SUA) concentrations. It is of interest to understand current prescribing practices.
Aims. To capture trends in the usage of allopurinol in a tertiary hospital and to explore options for improving therapy.
Methods. Data on allopurinol prescriptions and administrations from January 2008 to December 2012 were extracted from the e-prescribing system at St. Vincent's Hospital, Sydney. Estimated glomerular filtration rates (eGFR) and SUA concentrations were collated from pathology data.
Results. Allopurinol daily dosages ranged from 25 to 600 mg, where 38% and 51% of these doses were 100 mg and 300 mg, respectively. Mean±SD daily allopurinol dose for eGFR categories of <30, 30-60 and >60 mL/min/1.73m$^2$ were 122±63, 166±92 and 254±82 mg, respectively. SUA concentration was measured in 24% (n=486) of all patients, with a mean ± SD SUA concentration of 0.26±0.13mmol/L. Of all patients, 21% (n=348) had gout. SUA concentration was measured in 10% (n=35) of gout patients with a mean±SD SUA concentration of 0.38±0.13 mmol/L. The haematology and geriatrics departments made 30% (n=933) and 18% (n=570) of all allopurinol prescriptions.
Discussion. Renal function may still play a central role in guiding allopurinol initial dose rate at least. The SUA concentration in many gout patients did not meet recommended SUA targets (0.30-0.36 mmol/L). However, because only a small number of patients had SUA measurements this is not a fair representation. With increasing importance of SUA concentrations in allopurinol dose optimization in gout, mandatory SUA measurements in patients who are admitted on allopurinol may be warranted to monitor for effectiveness.

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**Pharmacokinetic studies in old age and frailty: The utilization of population modeling.**

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Introduction. Older adults are major users of medicines who often require dose adjustment due to age- or disease-related physiologic changes, intercurrent disease and polypharmacy. Understanding and characterizing variability is key to optimal dose selection in older people, especially those who are frail. In population pharmacokinetic (PopPK) analyses the population of patients (rather than individuals) is studied and data can be combined from individuals to determine average values.
Aims. The aim of this review was to investigate the utilization of PopPK studies in people over 65 years of age.
Methods. A MEDLINE and PubMed search identified 330 papers using the search term "population pharmacokinetics". Restrictions set on papers that describe research in were age group over 65 years; articles in English; published 2005-2013 (September); studies conducted in humans; and where full-text was available. 120 papers were excluded because they did not meet these restrictions.
Results. 210 papers were analysed. The median (range) number of patients in the studies was 100(5-3355), with most using NONMEM software for analysis and 86% investigated age as a potential covariate. There were only 21 studies that primarily focused on older people. The commonest drug class investigated was antimicrobials (9/21), most studies used sparse blood sampling methods and patients were generally acutely unwell. Some studies were translated into dosing guidelines specific for older people, although these were generally at a local level. No studies were identified on the use of PopPK to determine the impact of an objective measure of frailty on pharmacokinetics in older people. There were only 9 papers found (using some different search restrictions) that used some measure of frailty to investigate pharmacology in older people.

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**Do plasma oxypurinol concentrations provide a better fit to theoretical relationships than allopurinol dose?**

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Introduction. Allopurinol, prophylactically used in the treatment of gout, is a drug that is sub-optimally used as patients continue to experience acute gout attacks. Our mechanistic dose-response model (Eq1) has indicated that baseline (pre-allopurinol dosing) plasma urate concentrations ($U_P$) predict the maintenance dose of allopurinol required to achieve desired target urate concentrations ($U_T$), irrespective of renal function. $ID_{50}$ is the daily allopurinol dose reducing the inhibitable plasma urate by 50% and $U_R$ is the apparent resistant level of urate. We hypothesised that the plasma concentrations of oxypurinol, the active metabolite of allopurinol, may better predict plasma urate concentrations given the known variability of the pharmacokinetics of oxypurinol.

$$Eq1: U_T = \left(1 - \frac{D}{ID_{50} + D}\right) * (U_P - U_R) + U_R$$

$$Eq2: U_T = \left(1 - \frac{C}{IC_{50} + C}\right) * (U_P - U_R) + U_R$$

Aim. To compare the fits to the theoretical equations using allopurinol dose and plasma oxypurinol concentrations.

Methods. Patients with gout (n=22) received 1 to 4 increasing doses of allopurinol up to 600 mg/day (total doses=58). Plasma urate, creatinine and oxypurinol concentrations were recorded at each dose titration. The mean C values over a dosage interval were determined by a Bayesian program (TCIWorks). Data was fitted to the two equations by non-linear regression. For Eq2, $IC_{50}$ is the plasma concentration of oxypurinol reducing the inhibitable plasma urate by 50%.

Results. The dose-response equation provided a marginally better fit than the concentration-response equation (objective functions were 0.173 vs 0.185). Mean $ID_{50}$ and $IC_{50}$ values and 95% CI were 232 (133-418) mg and 11.9 mg/L (6.5-22.3) mg/L in the best-fit dose-response and concentration-response models respectively.

Discussion. Plasma oxypurinol concentrations do not improve the predictive properties of the dose-response equation relating the hypouricaemic response to the daily allopurinol dose.

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**Can we predict the optimum dose of allopurinol needed to effectively treat gout from any plasma urate?**

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Introduction. Allopurinol, prophylactically used in the treatment of gout, is a drug that is sub-optimally used as patients continue to experience acute gout attacks. A mechanistic dose-response equation indicated that the plasma concentrations of urate during allopurinol treatment ($U_T$) were determined from baseline (pre-allopurinol dosing) plasma urate concentrations ($U_P$), the dose of allopurinol (D), the $ID_{50}$ (dose of allopurinol which reduces the inhibitable urate by 50%) and an apparent allopurinol-resistant plasma concentration of urate ($U_R$). Model performance, however, has not yet been tested in patients who have already commenced allopurinol therapy.

$$U_T = \left(1 - \frac{D}{ID_{50} + D}\right) * (U_P - U_R) + U_R$$

Aims. To determine if the dose-response equation can be used to optimise dosage of allopurinol in patients who have already commenced allopurinol dosing.

Methods. Patients with gout (n=15) already taking allopurinol therapy were given increasing doses of allopurinol up to 600 mg/day (total doses=25). Plasma urate concentrations were recorded at each dose level. Plasma concentrations of urate during treatment ($U_T$) were predicted using the dose-response model and the mean optimised values ($ID_{50}$ = 226 mg, $U_R$ = 0.20 mmol/L) (Graham et al, 2013). At each successive dose titration, the $U_P$ was set as the plasma concentration of urate prior to an increase of allopurinol dose. The predicted $U_T$ was compared to the observed $U_T$ by a paired t-test and correlated against each other.

Results. The predicted $U_T$ correlated strongly with the observed $U_T$ when the incremental dose of allopurinol was fitted as the dose of allopurinol (D) in figure 1 ($R^2$=0.70). No significant difference was observed between the observed and predicted $U_T$ calculated in this manner (P=0.15).

Discussion. During allopurinol treatment, the incremental dose of allopurinol required to reach a subsequent target urate can be sufficiently predicted from any plasma urate concentration.

Graham GG et al (2013) Br J Clin Pharmacol DOI: 10.1111/bcp.12126.

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**Anticholinergic load in community dwelling elderly Australians with dementia**

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Introduction. Given that anticholinergic medications are associated with reduced cognitive function in the elderly (Bell et al, 2012), the prescribing of anticholinergics should be minimised in these patients and avoided, if possible in those with dementia.
Aims. To compare the loads of anticholinergic medications prescribed to Australian community dwelling elderly patients with and without impaired cognitive function.
Methods. Participants aged 75 years or older (n=1059), 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; Roth et al, 1986). A CAMCOG-R score of 79 or less was used as a relatively sensitive indicator of dementia. The number of mild to moderate anticholinergics, and the daily doses of these medications were examined in participants with and without dementia. Anticholinergic load was calculated using a weighting of 1-3 for mild to strong anticholinergics, and also multiplication of levels 2 and 3 drugs by a dosing factor (Carnahan et al, 2006).
Results. Approximately 60% of the dementia group (n=87) and 40% of the non-dementia group (n=972) were on at least one anticholinergic drug. The dementia group was exposed to a significantly greater anticholinergic load (determined by level) (1.47±0.20 *vs* 0.75±0.04; $P<0.001$). Values of anticholinergic load adjusted for dose were also significantly higher in the dementia group (1.70±0.28 *vs* 0.90±0.06; $P<0.005$).
Discussion. Anticholinergic load can exacerbate cognitive impairment and increase the possibility of adverse events in patients with dementia. These findings suggest there is scope for the reduction of anticholinergic load in community dwelling elderly Australians with dementia.

Bell JS et al (2012) Aust Fam Physician 41:45-48
Carnahan RM et al (2006) J Clin Pharmacol 46:1481-1486
Roth M et al (1986) Br J Psychiatry 149:698-709

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**Changes in polypharmacy, hyperpolypharmacy and exposure to anticholinergic and sedative medicines: A five-year study of community-dwelling older men**

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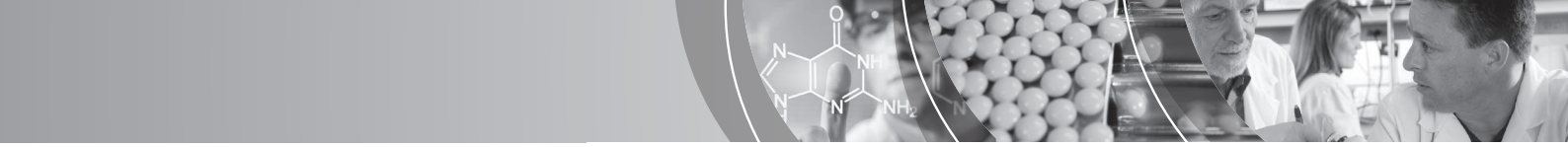
Introduction. Polypharmacy (≥5 medications), hyperpolypharmacy (≥10 medications) and Drug Burden Index (DBI) (medications with anticholinergic or sedative properties) exposures are common in older people and are associated with adverse clinical outcomes. Changes in medication exposures over time are not well described in older populations. Aim. To examine changes in polypharmacy, hyperpolypharmacy and DBI exposures in community-dwelling older men over 5 years. Methods. We used data from the Concord Health and Ageing in Men Project, a cohort of 1705 men aged ≥70 years living in the community at the time of recruitment (2005-2007). Participant information included sociodemographic characteristics, medical conditions, function measures and medication inventory at baseline, 2 years and 5 years. We compared the prevalence of exposure at baseline and year 5 using the McNemar's test. Results. At baseline, we studied 926 participants, for whom data was available at all 3 time points, were aged 75 (range 70-92) years, had 2 (range 0-8) comorbidities and 2.8% were frail. At baseline, 31.0% were exposed to polypharmacy, 2.5% to hyperpolypharmacy and 22.4% to DBI with statistically significant increases in all exposures at year 5. The prevalence of new exposure to any of the outcomes was 2-5 times greater than the prevalence of ceasing exposure at year 5. Discussion. Exposures to polypharmacy, hyperpolypharmacy and DBI were common and increased over 5 years in this cohort of older Australian men. Further research should investigate the implications of these changes on clinical outcomes in older people.

| | Polypharmacy | Hyperpolypharmacy | Drug Burden Index |
|---|---|---|---|
| | % (n) | | |
| Baseline prevalence | 31.0% (287) | 2.5% (23) | 22.4% (207) |
| Year 5 prevalence | 45.2% (419) | 7.5% (69) | 30.0% (278) |
| New exposure b/w baseline and Year 5 | 20.7% (192) | 6.2% (57) | 15.4% (143) |
| Ceased exposure b/w baseline and Year 5 | 6.5% (60) | 1.2% (11) | 7.8% (72) |



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**Dose individualization of the CYP3A substrate simvastatin by a midazolam microdose CYP3A activity measurement**

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Introduction. The vast majority of beneficial and adverse drug effects correlate with drug exposure as it is the case for simvastatin.
Aims. The dose of the CYP3A substrate simvastatin was to be individualised on the basis of CYP3A activity as assessed by midazolam metabolic clearance. This was also to be proven under multi-site inhibition with ritonavir.
Methods. In 18 healthy participants individual CYP3A activity was quantified using midazolam (dose 0.1 mg) metabolic clearance both alone and during CYP3A inhibition with 40 mg ritonavir. Thereafter, simvastatin acid exposure was determined after a simvastatin standard dose (40 mg) and doses adapted to individual CYP3A activity at baseline and during CYP3A inhibition.
Results. Midazolam metabolic clearance was highly variable (13.6-fold range: 377 - 5116 ml/min; geometric mean 973 ml/min) and a single oral dose of 40 mg ritonavir administered 10 min before midazolam significantly reduced the clearance by 82% (point estimate of 0.179 (90% CI: 0.155 – 0.207). After 40 mg simvastatin exposure of simvastatin acid was highly variable (30-fold range of $AUC_{0-24}$ from 11.4 to 343 h ng/ml (geometric mean 83.5 h ng/ml; CV=73%). Dose adaptation substantially decreased the variability of simvastatin exposure (CV=53%) with a dose range from 25 to mg. Individual dose adaptation during inhibition with ritonavir (40 mg) caused a 4.2-fold simvastatin $AUC_{0-24}$ increase despite a simvastatin dose range from 5 to 25 mg.
Discussion. Inter-individual variation in systemic exposure of simvastatin acid can be reduced by individually adapting its dose according to CYP3A activity thus confirming the validity of this strategy. The level of systemic simvastatin acid exposure might be influenced in the presence of co-medication by interactions at sites other than CYP3A activity like OATP1B1 inhibition, not represented by metabolic clearance of midazolam – an effect that could be captured by including the emerging knowledge in this field into calculations.

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**Understanding and improving metformin use in hospital.**

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Introduction. Metformin is the first-line treatment for type 2 diabetes mellitus. Metformin is eliminated very largely by renal excretion in the unchanged form and it is generally recommended that the dosage should be reduced with renal impairment and contraindicated in renal impairment (eGFR <30 ml/min) because excessive accumulation is considered to be associated with the serious adverse effect of lactic acidosis. There is, however, considerable discussion about this recommendation. Previously we have published (1) simulations of maximum daily doses of metformin based on renal function to ensure safe concentrations (peak <5 mg/L). Limited information on metformin use in hospitals is available.
Aim. To explore metformin use at St Vincent's Hospital (SVH) in relation to renal function.
Methods. The records of 20% of the patients receiving metformin (2008-2012) at SVH were analysed. Patients in four categories of eGFR were examined (<30, 30-60, 60-90 and >90 mL/min/1.73m$^2$). 9539 dosages of metformin were examined in 504 patients. The dose of metformin was categorised as appropriate if the dose was eGFR×33 ± 250 mg. Dosage above and below this range was categorised as high and low, respectively.
Results. The number of doses categorised (as low, appropriate and high) varied with the renal function; <30 mL/min/1.73m$^2$ (38, 17 and 129 doses), 30-60 mL/min/1.73m$^2$ (1366, 573 and 291 doses), 60-90 mL/min/1.73m$^2$ (3001, 624 and 215 doses) and >90 mL/min/1.73m$^2$ (2416, 865 and 4 doses) ($\chi^2$, P < 0.0001). Overall, 72% of all doses were categorised as low, 22% as appropriate and 7% as high.
Discussion. In patients with the lowest renal function, decreased dosage may often be required whereas increased dosage may be required in patients with better renal function. Monitoring of metformin plasma concentrations and detailed measurement of glucose control may assist in optimising dosage with metformin.

Duong et al (2013). Clin Pharmacokinet 52: 373-384.

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**Over-the-counter analgesic use by community patients in the Northern Territory, Australia**

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Introduction. Non-prescription analgesics constitute a significant proportion of over the counter (OTC) medicines worldwide. In Australia, analgesics are the second most commonly used OTC medication from community pharmacies. Recent concerns have been expressed on the potential for inappropriate use of OTC analgesics and patient's health risks due to unsafe medication. Researches into feasible methodologies for pharmacovigilance of OTC analgesics are therefore critically important.
Aims. The purpose of this article is to present the findings from qualitative study focused on community pharmacy patients and their experience and knowledge about appropriate use of non-prescription analgesics containing ibuprofen.
Methods. The study was conducted in four community pharmacies out of 22 in the metropolitan area of the Northern Territory, Australia. Patients presenting at the pharmacy were invited to complete a self-administered anonymous questionnaire during the study.
Results. A total 90 patients out of 90 (100%) completed the survey. 85.6% of patients were between 26-55 years of age, 2% aged 18-25 or > 65 years old, and 10% between 56-65 years. Their knowledge on the appropriate use of non-prescription ibuprofen, potential side effects and contraindications from use, and ibuprofen common indications was considered to be inadequate. Many patients reported they were on prescription medications (59%), had used ibuprofen for more than a year (68%), but did not seek medical advice before using non-prescription ibuprofen (57%).
Discussion. Many patients were unaware that non-prescription analgesics such as ibuprofen can cause potentially serious adverse effects when they are used inappropriately. Healthcare professionals have an important role in providing adequate patient information on non-prescription ibuprofen and promoting their safe use through education, monitoring and support.
Cusack L, de Crespigny C, Wilson C (2013) Health Soc Care Community 21:373-80.
Stosica R et al (2011) Int J Pharm Pract 19:236-45.

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**Garlic and skin cancer prevention - Preclinical perspectives**

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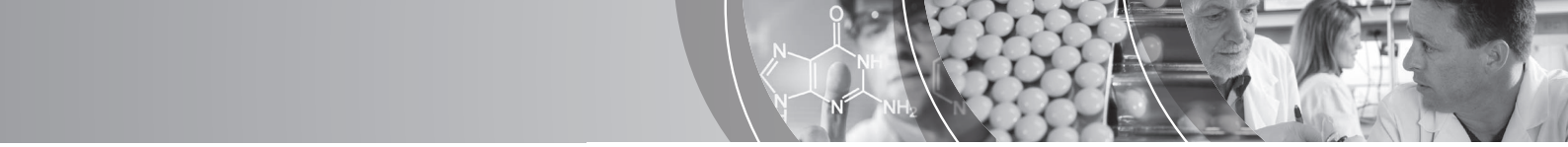
Introduction. Skin cancer is the most prevalent form of cancer in Australia. While melanomas are the most fatal form of the disease, non-melanoma skin cancers including basal cell carcinoma and squamous cell carcinoma are a source of significant morbidity and mortality. There is an increasing body of evidence to support the use of garlic (generally Allium vegetables/plants) and its active allyl sulphur constituents for skin cancer prevention.
Aims. The aim of the current study was to systematically review and evaluate the evidence derived from all types of studies published over the last 20 years that examined the protective effects of garlic and its active constituents on non-melanoma skin cancer.
Methods. The Medline and PubMed databases were searched for studies published in English, from August 1993 to August 2013. These studies examined the effects of garlic, and garlic constituents on non-melanoma skin cancer. The search terms used were: garlic, Allium Sativum, diallyl sulfide, diallyl disulfide, diallyl trisulfide, S-allyl cysteine, S-allyl mercaptocystein, allicin, ajoene, non-melanoma, skin cancer, chemoprevention, human, animal, randomized controlled trials, case control, cohort, in vivo, and in vitro studies.
Results. Literature evidence from animal and cell culture studies demonstrates the anti-skin cancer effects of two major active constituents of garlic, diallyl sulfide and diallyl trisulfide. In addition, the garlic-derived organosulfur component ajoene was reported to reduce tumour size in one clinical trial of patients with basal cell carcinoma. No evidence for other garlic constituents was found on non-melanoma skin cancer protection.
Discussion. There is consistent scientific evidence derived from animal and cell culture studies demonstrating protective effects of garlic on non-melanoma skin cancer. The reported UV-induced skin cancer protective properties were found to arise through the molecular changes in the multiple-stage process of skin tumour development and are dose related. In summary, the findings suggested that the different molecular targets modulated by garlic and its allyl sulphur constituents are useful indicators of success in future human non-melanoma skin cancer prevention trials.

Tilli CML et al (2003) Arch Dermatol Res 295:117-123
Wang H-C et al (2012) Ann NY Acad Sci 1271:44-52



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**Topical treatment with aspirin does not prevent non-melanoma skin cancer in mice**

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Introduction. Non-melanoma skin cancer (NMSC) is a common cancer type with increasing incidence. Preventive methods include standard sun protection behaviour such as avoiding midday sun, seeking shade, sun-protective clothing, and sunscreen lotion. As total avoidance of sun exposure would seem unrealistic, other interventions to prevent NMSC are needed. Earlier studies have shown that the use of non-steroid anti-inflammatory drugs (NSAIDs), particularly aspirin, seems to have beneficial effects in patients with NMSC squamous cell carcinoma.
Aims. The aim of the current study was to evaluate topical application of aspirin, and other non-steroidal anti-inflammatory drugs as chemoprevention in a mouse model of ultraviolet light-induced skin tumours, since these agents have been reported to have tumour inhibiting properties.
Methods. One hundred mice were treated with UVA+B radiation followed by chemoprevention or placebo. Animals were irradiated with daily doses of UV for approximately 10 min per day, 5 days per week for 10 weeks. After this 10-week, there was no further UV-exposure. The integrated UV-A irradiance (280-320 nm) was 2.4 X 10-4 W/cm2 and the UV-B irradiance (320-400 nm) was 1.8 X 10-3 W/cm2. Mice were divided into 5 groups (n=20 per group). Group 1 was treated with methanol (placebo); Group 2 received 2% aspirin in methanol; Groups 3,4,5 received 2% indomethacin, paracetamol, or flurbiprofen in methanol respectively. Mice were killed after 35 weeks.
Discussion. There were no significant effects of the aspirin treatment with respect to presence of skin tumours, number of tumours, or tumour size. Aspirin was ineffective as chemoprevention in the dose regimens used in this study. Compared to aspirin and placebo, topical treatment with other NSAIDs was found to be more effective.

Torti DC et al (2010) J Am Acad Dermatol 65:304-312
Johannesdottir SA et al (2012) Cancer 118:4768-4776

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**Consortium for clarity in medicine labelling**

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Introduction. Labelling reform for all medicines is a key element to support quality use of medicines. Many preventable harms and hospital admissions are contributed to by medicine names and aspects of labelling. ASCEPT, and other organisations, made a submission to the TGA request for comments on their Review on Labelling and Packaging.
Aims. To progress action on labelling and convene a representative health professional consortium to lobby effectively for changes to medicine labelling to enhance consumer health outcomes.
Methods. A series of principles was drafted, and a position paper developed in consultation with key professional groups.
Results. A position statement was drawn up, with agreement from seven key professional groups, enunciating key points of agreement. The position statement was presented in February 2013, face to face, to senior members of the Therapeutic Goods Administration responsible for driving their Review. Initial steps to enhance safety and reduce confusion were proposed around the most important naming and typographical problems in medicine labels.
Discussion. The position statement was well received and the major suggested 'first steps' were taken to, and endorsed by, an ensuing meeting of the TGA External Reference Group on labelling. In particular the following changes were recommended to be implemented as soon as possible: - the active ingredient should be the primary identifier for all medicines; - the active ingredient name should be as close as possible to any brand name on all areas of packaging; - initially these changes should be applied to generic products. The consortium recommended that for generic products the active ingredient name should be above any brand or sponsor name, in larger font and in bolder font. An embargo on invented brand names, prefixes and suffixes on new generic registrations was also recommended. We await changes to regulations from the recommendations of the consortium.

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**Prevalence and factors associated with statin use in geriatric oncology**

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Introduction. With increasing age and reduced life expectancy the harms of long-term preventative medications may outweigh the benefits. There is minimal evidence that statins reduce mortality in people aged ≥80 years. Recent evidence suggests statins are associated with increased pain and musculoskeletal conditions.
Aim. To investigate the prevalence and factors associated with statin use in people aged <80 years and ≥80 years.
Methods. Between January 2009 and June 2010, 391 patients attended the medical oncology outpatient clinic at the Royal Adelaide Hospital, and were assessed under the oncogeriatric program. Of these, 106 were aged ≥ 80 years. Each patient completed measures of pain (10 point visual analogue scale [VAS]), medication use and comorbidities. Unadjusted and adjusted logistic regression was used to compute adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association between statin use and clinical parameters.
Results. The point prevalence of statin use was 34.7% (n=99) in people aged <80 years and 38.7% (n=41) in people ≥80 years. After adjusting for age, gender and Charlson's comorbidity index, statin use was associated with higher prevalence of patient self reported pain (VAS ≥5) than non-use (OR 3.27 [95% CI 1.03-10.4]) in people aged ≥80 years, but not in people <80 years. There were 41 statin users aged ≥80 years, with 71% (n=29) using statins for primary prevention. Of those 55% (n=16) had a palliative treatment intent. Statin use for secondary prevention accounted for 29% (n=12) of statin users, with 67% (n=8) having a palliative treatment intent.
Discussion. The prevalence of statin use was similar in people aged <80 years and ≥80 years, with statin use associated with self-reported pain in people aged ≥80 years. This highlights the potential for deprescribing statins in the geriatric oncology setting, especially in those with palliative treatment intent.

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**Factors associated with buprenorphine abuse among clients seeking treatment in Finland**

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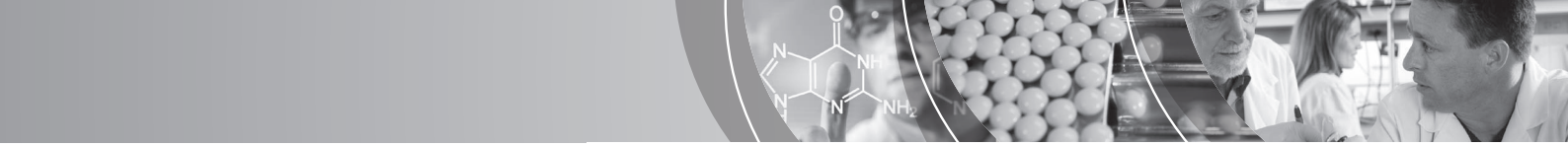
Introduction. The abuse of prescription opioids including buprenorphine is becoming increasingly widespread in North America, Australia, Europe and South Asia. Among prescription opioid users there are distinct groups differing from each other in key characteristics such as age, education, health status and treatment history. These characteristics may be associated with successful treatment outcome. The characteristics of those who abuse buprenorphine are not well established.
Aims. This study examined the social, health and treatment-related factors associated with buprenorphine abuse in Finland.
Methods. Clients who sought treatment from the Helsinki Deaconess Institute between January 2001 and August 2008 participated in structured clinical interviews. Clients who reported that buprenorphine was their primary drug of abuse (n=670) were compared to clients who reported amphetamine as their primary drug of abuse (n=557). Logistic regression was used to compute unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (CIs) for factors associated with buprenorphine compared to amphetamine abuse.
Results. Of 670 buprenorphine clients, 82 (12.5%) were employed, 503 (83.0%) relied on social benefits and 499 (77.4%) reported elementary school or lower level of education. In multivariate analyses, buprenorphine abuse was associated with male gender (OR 1.57, 95% CI 1.17-2.09), daily abuse (OR 5.45, 95% CI 4.14-7.18), no drug free months during the last year (OR 1.68, 95% CI 1.23-2.29), self-referral to treatment (OR 1.33, 95% CI 1.01-1.75), referral to outpatient treatment (OR 1.40, 95% CI 1.00-1.93), absence of psychotic symptoms (OR 0.33, 95% CI 0.24-0.45), and inversely associated with increasing age (OR 0.95, 95% CI 0.93-0.97 per year).
Discussion. Despite more intense abuse patterns, clients seeking treatment for buprenorphine abuse shared similar social and health-related characteristics to amphetamine clients. These characteristics were different to characteristics of prescription opioid users in previous studies. These characteristics should be taken into consideration when developing and targeting intervention programs.



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**Monitoring the anti-*Helicobacter Pylori* activity of some nutritional antibiotics *in vivo*, using the MetAtron/Hunter**

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Introduction. Some pathogenic microorganisms (PM) are difficult to culture *ex vivo* for antibiotic screening. By locating unique bio-resonance frequencies of viable PM *in vivo*, it may be possible to identify their susceptibility or resistance to selected antibiotics.
Aims: To test the susceptibility of gastric *H.pylori* to antibiotics in foodstuffs and sterilised water.
Methods. The MetAtron (developed by IPP) can non-invasively detect and semi-quantify PM infestations within the gastro-intestinal tract including gastric *H.pylori*. Nutritional supplements with antibacterial and anti-arthritic activity were taken by volunteers (with their informed consent) twice a day for five days, after establishing reproducible levels of infection. Response was monitored two days after the last dose. For comparison, metronidazole and nanoparticulate metallic silver (NMS) were taken separately by other volunteers also infected with *H.pylori*.
Results. Culinary preparations of *Nigella sativa*, black cumin oil (Khan 1999) and some OTC celery seed products (Zhou et al 2009) did suppress *H.pylori* but not as efficiently as an NMS preparation (Lunasol$^{R}$) used for sterilising water. Metronidazole (400 mg tid, 7 days) given alone was not effective over this short time period.
Discussion. With appropriate Ethical approvals, it is feasible to rapidly check the potency and duration of antimicrobial activities of selected dietary components against pathogens within the GI tract. Normal volunteers without overt disease (gastritis, peptic ulcers) may still be suitable for some pilot studies. This strategy has proved useful for locating anti-*Proteus* activity in the bladder and large bowel (Whitehouse et al 2012). The MetAtron can be a useful tool for linking Ancient Wisdom to modern medicine.

Khan MA (1999) Inflammopharmacol 7:15-35
Zhou Y et al (2009) J Pharm Pharmacol 61;1067-1077

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**Polypharmacy and medication regimen complexity as predictors of hospital discharge directly to home: a cohort study**

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Introduction. Older people often take multiple medications and are at high risk of adverse events. It is a policy priority to facilitate older people to stay at home longer. No previous studies have investigated whether medication regimen complexity is associated with hospital discharge directly to home.
Aims. To investigate the association between polypharmacy and medication regimen complexity with hospital discharge directly to home versus discharge to non-community settings.
Methods. The study comprised patients aged ≥70 years consecutively admitted to the Geriatric Evaluation and Management Unit at The Queen Elizabeth Hospital between October 2010 and December 2011. Medication regimen complexity at hospital discharge was calculated using the 65-item validated Medication Regimen Complexity Index (MRCI). Logistic regression was used to compute adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for medication-related factors associated with discharge directly to home versus non-community settings (rehabilitation, transitional care, and residential aged care).
Results. From 163 eligible patients, 87 were discharged directly to home (mean age 84.6, SD6.9; mean MRCI26.1, SD9.7) while 76 were discharged to non-community settings (mean age 85.8, SD5.8; mean MRCI29.9, SD13.2). After adjusting for age, gender, comorbidity and activities of daily living (ADL), having a high medication regimen complexity (MRCI>35) was inversely associated with discharge directly to home (OR0.26, 95% CI 0.12-0.60) whereas polypharmacy (≥9 medications) was not significantly associated with discharge directly to home (OR 0.94, 95% CI 0.47-1.87). The ORs were unchanged when medications prescribed for a short duration at the time of hospital discharge where excluded when calculating regimen complexity.
Discussion. Having a high medication regimen complexity was inversely associated with discharge directly to home, while polypharmacy was not associated with discharge destination. This highlights the potential importance of simplifying medication regimens in older people discharged from hospital.

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**The influence of CYP2C9 and VKORC1 genotype on the predictive performance of a Bayesian forecasting method for warfarin therapy**

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Introduction. Genetic differences in warfarin metabolism (via CYP2C9) and vitamin K recycling (VKORC1) have been found to significantly influence warfarin maintenance dose requirements. A Bayesian forecasting method for warfarin therapy should predict dosing requirements without prior genetic data.
Aims. The aim of this study was to determine the influence of CYP2C9 and VKORC1 genotype on the predictive performance of a Bayesian warfarin dosing method.
Methods. Patients who were initiating warfarin therapy were genotyped for *CYP2C9 *1*, *2* [rs1799853] and *3* [rs1057910] alleles and for the VKORC1 promoter variant *-1639G>A* [rs9923231]. The warfarin dosing history for each patient was retrospectively recorded up to the steady-state INR (INRss). TCIWorks, a Bayesian dose individualisation tool, was used for predicting maintenance doses under two scenarios; 1) using feedback from the first 4 INR measurements after the initiation of therapy, and, 2) using all INR measurements up to the last dose change. Observed and predicted maintenance doses were compared using measures of bias (mean prediction error [MPE]) and imprecision (root mean square error [RMSE]).
Results. A total of 46 patients completed the study. Maintenance dose predictions were positively biased for patients with VKORC1 G/G genotype using the first 4 INRs (MPE +1.0 mg/day [95% CI 0.4, 1.7]) and using all INRs (MPE +1.1 mg/day [95% CI 0.4, 1.9]). The 95% CI of the MPE included zero for patients with CYP2C9 *1*1, *1*2 and *1*3 genotypes and for VKORC1 A/G and A/A genotypes indicating unbiased predictions.
Discussion. Warfarin maintenance dose predictions were over-predicted by 1mg/day on average in patients with VKORC1 G/G genotype and were less precise than other VKORC1 genotypes. This did not improve with the addition of the complete INR response history. Further research to explore the influence of G/G genotype on Bayesian dosing predictions is warranted.

Wadelius M et al (2009) Blood 113:784-792
Wright DFW & Duffull SB (2013) Clin Pharmacokinet 52:59-68

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**SimCYP assessment of population level inter-individual variability in olanzapine clearance.**

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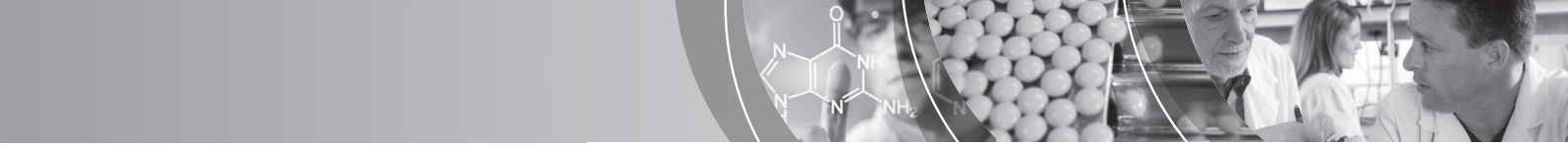
Introduction. Olanzapine (OLZ) is an atypical antipsychotic used in the management of schizophrenia and related psychosis. There is wide inter-individual variability in OLZ pharmacokinetics and response, and the factors that contribute to this variability remain poorly characterised. Thus, at a population level, co-variates that that influence the clearance of OLZ, and subsequently maintenance dose required to attain a therapeutic steady-state concentration, are poorly understood.
Aims. To assess the inter-individual variability in OLZ clearance predicted from *in vitro* kinetic data utilising physiological-based pharmacokinetic (PBPK) modelling.
Methods. The SimCYP population-based ADME Simulator was used to predict the steady-state clearance for a 10mg dose of OLZ based on *in vitro* kinetic data utilising recombinant enzymes in the presence of albumin. Liver intrinsic clearance ($CL_{int.liver}$) was calculated from *in vivo* pharmacokinetic studies where a 10mg dose was studied (n=5) and compared to the liver intrinsic clearance ($CL_{int.liver\cdot sim}$) of PBPK simulations. Trough OLZ concentrations (n=170) at steady-state were collated from the Flinders Medical Centre therapeutic drug monitoring (TDM) service, and data from the patient cohort were compared with the results from the virtual population.
Results. PBPK simulations for OLZ clearance were comparable to reported data from *in vivo* pharmacokinetic studies in healthy volunteers both in terms of population mean and variability. The mean $CL_{int.liver}$ obtained from *in vivo* pharmacokinetic studies was 69.85L/hr (range 43.39-126.70L/hr) versus a mean $CL_{int.liver.sim}$ obtained from PBPK simulations of 74.11L/hr (range24.43-151.86L/hr). Cumulative frequency plots of trough OLZ concentration at steady-state were comparable between the patient cohort and the virtual population.
Discussion. PBPK modelling utilising the SimCYP Population-based ADME Simulator facilitated the assessment of inter-individual variability in OLZ clearance. This approach demonstrated good correlation with both single dose pharmacokinetic studies and population data obtained from the TDM service.



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**Metformin in haemodialysis patients with diabetes: short term safety and glycaemic effects**

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Introduction. Metformin is not used for type 2 diabetes (T2DM) in chronic kidney disease because of fears of lactic acidosis. Metformin is renally cleared and its clearance decreases in proportion to decreasing renal function (Graham et al, 2011). In mild and moderate renal impairment dose reduction, proportional to renal function, can maintain metformin plasma concentration in the therapeutic range and avoid toxicity. This has not been studied systematically in severe renal impairment. We hypothesised that metformin can be safely used in patients on haemodialysis if dosed based on predicted drug clearance.
Aim. To examine the pharmacokinetics and safety effects of metformin in patients with T2DM on haemodialysis.
Method. An assay for metformin quantification using Ultra Performance Liquid Chromatography- Mass Spectrometry (UPLC-MS) was developed. Six patients with T2DM on haemodialysis were given 500 mg metformin orally post dialysis for three weeks, (1.5g weekly, about 10% of the usual dose) with frequent sampling for metformin measurements. Lactate and bicarbonate concentrations were measured at baseline, at expected metformin $T_{max}$ and weekly during therapy.
Results. Preliminary analysis of the pharmacodynamic data show a rise in lactate of 0.68 ± 0.12 mmol/L (mean ± SD) from baseline while on metformin treatment. The highest single lactate concentration was 3.75 mmol/L. Bicarbonate concentration exhibited a mean rise 2.58 ± 2.25 mmol/L. Standard curves for the UPLC-MS assay were linear over the concentration range of 0.25-5 mg/L, and intra- and inter-day coefficients of variation were <12%. Analysis of plasma metformin concentrations using the assay is currently underway.
Discussion. Metformin dosing based on predicted metformin clearance in dialysis may allow for safe metformin use in patients on haemodialysis.

Graham GG et al. (2011) Clin Pharmacokinet 50(2):81-98.

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**The subchronic phencyclidine rat: modelling the pathophysiology of schizophrenia and reversal with the antipsychotic, risperidone.**

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The development of animal models for schizophrenia has proven difficult, some symptoms have exclusively 'human' characteristics and thus unsuited, while other behaviours have been modelled but cannot be associated with concurrent brain neurochemical changes. Persistent blockade of NMDA receptor function by repeated phencyclidine (PCP) produces pathophysiological changes that model the cognitive/attentional deficits, social dysfunction and pathophysiological dysfunction of parvalbumin containing GABAergic neurons, observed in schizophrenia(1,2). In this study we evaluate the validity of the sub-chronic PCP rat in modelling behaviour associated with the positive symptoms of schizophrenia, and the effect of the antipsychotic, risperidone on the this behaviour.
Twenty-four (n=8/group) male Lister-hooded rats were administered PCP at a dose of 2mg/kg i.p. bi-daily for 1 week, or vehicle. Half of the phencyclidine group was concurrently treated with risperidone (0.5mg/kg i.p.) twice daily for 15 days, beginning 3 days before the start of PCP administration. Six weeks later all rats received a single PCP (3.2mg/kg i.p.) challenge and were placed in a locomotor box for 20minutes where their activity was recorded.
The PCP group displayed significantly more activity compared with the CON group after PCP challenge. Co-administration of the antipsychotic risperidone significantly reduced the effect of the PCP challenge after bi-daily PCP administration. Group effect: $(F(2,22)=25.9; p<0.0001)$; PCP v PCP&Risp $p<0.05$.
PCP produces a long-lasting behavioural sensitization which may be associated with neuronal toxicity or receptor sensitization. This effect is attenuated by co-administration of the atypical antipsychotic, risperidone, suggesting that risperidone may have some neuroprotective action against chronic PCP treatment.

1. Mc Kibben et al., (2010) Behavioural Brain Research, 208-132-136.
2. Jenkins et al. (2008) Behavioural Brain Research, 194, 230-235.

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**The Direct Action of Cannabidiol at GABA-A Receptors**

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Introduction: Cannabinoids act upon the cannabinoid receptor system, however, this may be overly simplistic as endogenous and synthetic cannabinoids have been shown to act at various GABA-A receptors. The actions of phyto-cannabinoids have not been well elucidated at these inhibitory receptors.

Cannabidiol (CBD) is considered to be the major non-psychoactive component of cannabis. However, it has been found to possess anxiolytic, anti-epileptic and anti-psychotic properties in human trials. These properties may be suggestive of GABAergic involvement. Additionally, CBD has been shown to act upon $\alpha_3$subunit containing glycine receptors. Both glycine and GABA-A receptors are chloride gated ion channels and part of the Cys-loop receptor super-family. As such, we present a study on the direct action of cannabidiol (CBD) at specific GABA-A receptor combinations.

Aims: To assess for direct activation and modulation of $\alpha_1\beta_2\gamma_{2L}$, $\alpha_1\beta_3\gamma_{2L}$, $\alpha_2\beta_2\gamma_{2L}$ and $\alpha_4\beta_3\delta$ GABA-A receptors by CBD and to contrast this to 2-arachidonyl glycerol (2AG), the major central endo-cannabinoid.

Methods: Recombinant DNA techniques and two-electrode voltage clamp electrophysiology of receptors expressed in *Xenopus laevis* oocytes.

Results: CBD and 2AG were weak partial agonists at $\alpha_1\beta_2\gamma_{2L}$, $\alpha_2\beta_2\gamma_{2L}$ and $\alpha_4\beta_3\delta$GABA-A receptors. However, significant direct activation was observed with CBD at$\alpha_4\beta_3\delta$ receptors, where CBD was 33% as efficacious as GABA, while10$\mu$M CBD saw 10.4% of the maximum GABA currents observed at $\alpha_4\beta_3\delta$ receptors.

CBD and 2AG were found to modulate GABA significantly, with CBD being the more efficacious compound. At the physiologically relevant dose of 10$\mu$M CBD, the modulation of a GABA $EC_{5-10}$upon $\alpha_4\beta_3\delta$, $\alpha_1\beta_2\gamma_{2L}$ and $\alpha_2\beta_2\gamma_{2L}$ receptors was 273%, 234% and 217% respectively.

Discussion: This research has found CBD to modulate and/or directly act at$\alpha_1\beta_2\gamma_{2L}$, $\alpha_1\beta_3\gamma_{2L}$, $\alpha_2\beta_2\gamma_{2L}$ and $\alpha_4\beta_3\delta$GABA-A receptors. In addition, CBD has been found to generally modulate these receptors more efficaciously than 2AG, the major central endo-cannabinoid.

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**Social and anxiety-like behaviours in mice with a genetic deletion of insulin regulated aminopeptidase**

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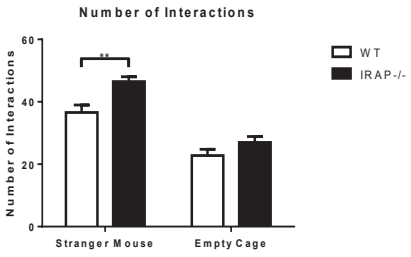


Introduction. *In vitro* assays have established that the enzyme insulin regulated aminopeptidase (IRAP) has the ability to metabolise the neurohypophysial hormone, oxytocin (Matsumoto *et al*., 2000). Furthermore, oxytocin has been shown repeatedly to act as a prosocial and anxiolytic neuromodulator when administered centrally (Kirsch *et al*., 2005), and is in early stage clinical trials as a treatment for autism.

Aims. The present study investigated the social and anxiety-like behaviours of mice with a genetic deletion of the enzyme IRAP.

Methods. Social behaviour in mice was tested using a social approach three chamber test, while anxiety-like behaviours were measured with the elevated plus maze and the open field.

Results. Social behaviour to increased in the IRAP knockout group compared the wild-type group with a main genotype effect observed in the number of interactions with the stranger mouse (n=16-21, P= 0.0006), figure shown. Genotype had no effect on anxiety-like behaviours in both the elevated plus maze and open field

Discussion. This study is the first to investigate IRAP as a novel target for sociability through its effects on the oxytocinergic system. It supports previous studies in IRAP knockout mice that found no change in anxiety-like behaviours. Ultimately, this study indicates that IRAP inhibition may be to a novel method to target the oxytocinergic system centrally to promote pro-social behaviour.

Kirsch P et al (2005) J. Neurosci 25: 11489-11493

Mastumoto H et al (2000) Eur. J.Biochem. 267 46-52

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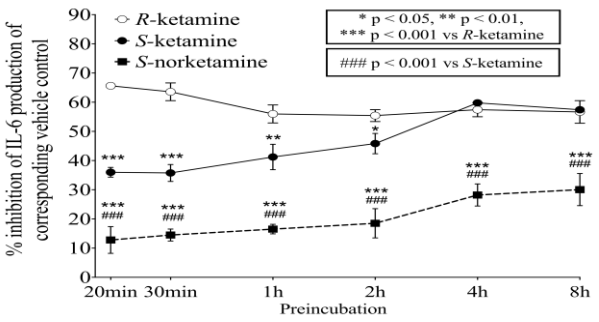
**Ketamine and its metabolite inhibit lipopolysaccharide (LPS)-induced interleukin-6 production in a time- and concentration-dependent manner: potential involvement of multiple pathways**

Yibai Li[1], Mark R Hutchinson[2], Janet K Coller[1] and Andrew A Somogyi[1], Discipline of Pharmacology, The University of Adelaide[1], Adelaide, SA; Discipline of Physiology, The University of Adelaide[2], Adelaide, SA

Introduction. Racemic ketamine inhibits post-operative interleukin-6 (IL-6) inflammatory response, possibly via the suppression of Toll-Like receptor 4 (TLR4) signalling. However, the contribution of each individual enantiomer and their corresponding active metabolites, *S*- and *R*-norketamine, to such anti-inflammatory activity is unclear.

Aim. To examine the effect of ketamine and norketamine enantiomers on LPS-induced IL-6 production using human embryonic kidney 293 cells stably expressing human TLR4 and co-signalling molecules.




Methods. The LPS-induced IL-6 concentrations after pre-incubation with ketamine and norketamine enantiomers at different concentrations (1, 10, 100 μM) and exposure time (20 min to 8 h) were quantified using ELISAs. The time-course response of ketamine on IL-6 production exposure was also examined by removing the supernatants at the end of pre-incubation prior to LPS stimulation.

Results. The inhibitory effects of ketamine and norketamine on induced IL-6 production were concentration-dependent (100 > 10 > 1 μM). Time-course effects on IL-6 inhibition were observed for S-norketamine and both ketamine enantiomers (inset figure). The IL-6 production was inhibited by both ketamine enantiomers when drug was removed from supernatants after 4 h pre-incubation ($p < 0.0001$) but not after 20 min pre-incubation ($p > 0.34$).

Discussion. The time course and stereoselective difference in inhibition of LPS-stimulated IL-6 production by ketamine and norketamine enantiomers may reflect a mechanistically-based difference between the acute and long-term anti-proinflammatory activity of ketamine. The mechanism of acute activity is likely associated with inhibition of TLR4 binding, whereas the long-term activity appears to be independent of TLR4 receptor binding.

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**The effect of quetiapine (Seroquel™) on conditioned place preference and elevated plus maze tests in rats when administered alone and in combination with (+)-amphetamine**

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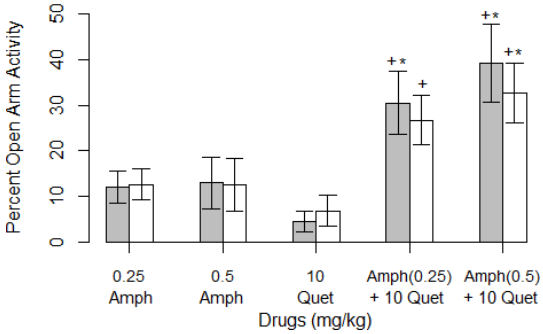
Introduction. Recent case reports describe drug-seeking behaviour in humans to obtain and recreationally use and abuse quetiapine (Quet), a clinically-used atypical antipsychotic, alone and in combination with (+)-amphetamine (Amph).

Aims. To determine whether administration of Quet, with and without Amph, to rats affects the reward measured by conditioned place preference (CPP) and/or decrease anxiety as measured in the elevated plus maze (EPM).




Methods. Amph (0.25, 0.5 mg/kg), or Quet (10 mg/kg) was administered either alone or in combination to rats tested for CPP followed by the EPM test.

Results. Combining 0.5 mg/kg Amph and 10 mg/kg Quet significantly increased percentage open arm entries (% OAE, white bars) and time (% OAT, grey bars) compared to 0.5 mg/kg Amph alone (*P< 0.05) or 10 mg/kg Quet alone ($^{+}$P< 0.05). The 0.25 mg/kg Amph and 10 mg/kg Quet group was significantly different from 10 mg/kg Quet alone in % OAT and % OAE ($^{+}$P< 0.05) and from 0.25 mg/kg Amph alone in %OAT (*P< 0.05). Quet (10 mg/kg) alone produced no CPP and reduced 0.25 mg/kg Amph-induced CPP, but not 0.5 mg/kg Amph-induced CPP.

Discussion. The Quet-induced anxiolytic effect in the EPM might explain why humans are misusing Quet and combining it with Amph. It is possible that humans experience an anxiolytic effect of the combined drugs and relatively unaltered rewarding effects of Amph. The results give some explanation as to why humans are abusing and misusing Quet, despite its dopamine $D_2$ receptor antagonism; future studies are needed to identify the pharmacological mechanism mediating this behaviour.

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**Co-administration of the phytocannabinoid CBD modulates the neurobehavioural effects of acute and repeated THC exposure in mice**

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Introduction. Australian cannabis contains the highest concentrations of psychotropic delta9-tetrahydrocannabinol (THC) in the world, but is virtually devoid of cannabidiol (CBD). As CBD may protect against the adverse effects of THC, this is a cause for concern. Animal studies addressing the interactive effects of THC and CBD have largely focussed on acute administration studies with CBD doses far higher than THC, which historically at best reaches a 1:1 CBD/THC dosing ratio in cannabis.
Aims. Here we aim to address whether co-administered CBD modulates the acute and repeated neuronal, behavioural and physiological effects of THC in mice.
Methods. Male mice were injected with vehicle, THC, CBD or THC and CBD in combination at 10 mg/kg daily over 15 days. Following acute dosing, brain activation was assessed using c-Fos immunohistochemistry. Animals were examined for cannabinoid-induced hypothermia and locomotor suppression every other day for 15 days. On days 1 and 15, mice were tested for prepulse inhibition of startle (PPI), a measure of sensorimotor gating disrupted in schizophrenia. Long-term neuroadaptive changes to repeated cannabinoid exposure was examined using ΔFosB/FosB immunohistochemistry.
Results. Acutely, CBD potentiated hypolocomotion and antagonised hypothermia induced by THC. Further, CBD blunted acute THC-induced PPI facilitation. Repeated THC promoted behavioural sensitization where locomotor suppression developed into locomotor hyperactivity over days 3 to 5, but became indistinguishable from vehicle animals at day 15. CBD suppressed this THC-induced sensitization. Acute CBD appeared to offset c-Fos expression in the hippocampus and periaqueductal gray. However, repeated cannabinoid dosing didn't alter ΔFosB expression.
Discussion. CBD modulated the acute and repeated neurobehavioural effects of THC in a way that varied dependent on the measured variable. Our results showing CBD offsets THC-induced behavioural sensitization may be relevant to improving the understanding of the ability of CBD to protect against addictive and propsychotic effects of THC.

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**Genetic, pathological and physiological determinants of transdermal fentanyl pharmacokinetics.**

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Introduction: The strong opioid analgesic fentanyl delivered transdermally targets patients with stable opioid requirements, including cancer patients. Fentanyl is mainly metabolised to norfentanyl (inactive) by CYPs 3A4 and 3A5, and dose requirements vary greatly. We hypothesised that *CYP3A4* and *CYP3A5* mutations contribute to variability in fentanyl metabolism to norfentanyl, and thus serum fentanyl concentrations for a given dose.
Aim: To investigate if CYP3A4/5 genetic variants, together with clinical and patient factors, influence serum fentanyl and norfentanyl concentrations and their ratio, in cancer pain patients receiving transdermal fentanyl.
Methods: *CYP3A4*22* and *CYP3A5*3* polymorphisms were analysed in 620 cancer pain patients from the European Pharmacogenetic Opioid Study receiving transdermal fentanyl (12.5-700 μg/h). Using stepwise linear regression, genetic variability was examined in combination with patient pathological and physiological factors, for their association with serum fentanyl concentrations and metabolic ratio (norfentanyl:fentanyl).
Results: Serum fentanyl concentrations (0.09-234 nM) and metabolic ratios (0.08-499) varied substantially. *CYP3A4*22* and *CYP3A5*3*, CYP3A inhibitor co-administration, and variables related to organ function (GFR, body mass index (BMI), kidney disease, age, serum albumin), accounted for 14% of variability in metabolic ratio. However, *CYP3A4*22* and *CYP3A5*3* were not associated with serum fentanyl concentrations. Only 45% of variability in serum fentanyl concentrations was accounted for by dose alone, and only 47% in total on addition of other clinical variables (sex, CYP3A inhibitor co-administration, BMI, serum albumin).
Discussion: Fentanyl serum concentrations and metabolic ratio vary substantially between cancer pain patients on transdermal fentanyl patches. *CYP3A4*22* and *CYP3A5*3* genotypes, and multiple clinical factors, combine to influence transdermal fentanyl pharmacokinetics, but accounted for only a small proportion of variability in this study. Identification of remaining factors determining serum fentanyl concentrations, and their relationship to efficacy and adverse effects, may aid in improving the safety and effectiveness of transdermal fentanyl.

**Does a transport defect underlie abnormal pharmacokinetics for the majority of 5-fluorouracil toxicity cases?**

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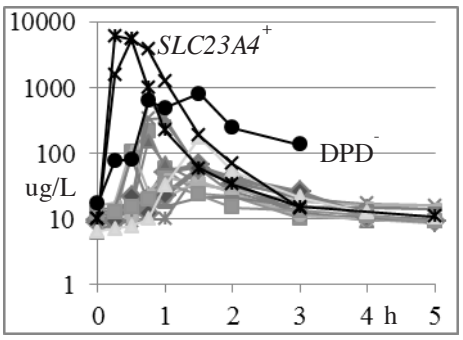
Introduction. About 1/3 of severe side effects during cancer chemotherapy with 5-fluoro-uracil (5FU) or capecitabine results from partially deficient dihydropyrimidine dehydrogenase (DPD), the major catabolic step for 5FU. The underlying cause of the remaining 2/3 of toxicity has remained unknown. The candidate gene for 5FU uptake in mammals is *SLC23A4* (*SNBT1*) but it is inactivated in humans by a large deletion.

Aim. To develop a test to predict 5FU toxicity and identify the causative gene(s).

Methods. Twelve healthy male adults and six patients who had severe 5FU toxicity were given 250 mg thymine (5-methyl-uracil) orally. Timed plasma, urine and saliva samples were collected, and analysed by HPLC-MS/MS for thymine and its successive catabolites dihydrothymine and ß-ureidoisobutyrate. Takara Long-range PCR kits were used to amplify *SLC23A4*.

Results. Fig.1 shows thymine was quickly absorbed into plasma by healthy subjects (grey), mean Cmax= 170 (range 34-679) ug/L; estimated clearance (Cl/F) had a broad range and in excess of liver blood flow (mean 57.9, range 9.30-175 L/h/kg). Basal plasma dihydrothymine was ~9-fold higher than thymine; both dihydrothymine and ß-ureidoisobutyrate exhibited formation-rate limited kinetics. Delayed thymine clearance in two patients (Fig.1, filled circles) was consistent with DPD-deficiency, confirmed genetically. However, three other patients exhibited rapid thymine uptake and plasma Cmax values up to 30-fold above average (mean 4190, range 3000-6190 ug/L) (Fig.1, crosses). Control long-range PCRs had the expected short (6kb) amplicon, but the rapid thymine uptake phenotype produced a full-sized amplicon (12kb).




Discussion. The thymine load test easily distinguished DPD deficiency from control DPD. A hitherto undescribed 'rapid thymine uptake' PK phenotype was discovered in three of six patients, with a putative non-deleted form of the *SLC23A4* transporter coinciding with this abnormal PK. We hypothesise a 'primal' active form of *SLC23A4* is polymorphic among humans, underlying the majority of fluoropyrimidine toxicity cases.

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**Selected polymorphisms in the CLPTM1L gene are associated with lung cancer risk in Han Chinese population**

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Introduction. Genome-wide association studies have implied the association of inherited genetic variants in different loci of chromosome with lung cancer risk in smokers and non-smokers, including the cleft lip and palate transmembrane 1-like (CLPTM1L) gene at 5p15.33. (Timofeeva et al 2012)
Aims. We carried out a case-control study to investigate the association of polymorphisms of CLPTM1L gene and lung cancer risk in the Han Chinese population.
Methods. Nine tag single-nucleotide polymorphisms (tSNPs) in the CLPTM1L gene with minor allele frequency >5% in the HapMap CHB population were selected and genotyped for 309 cases of lung cancer and 310 healthy controls (male/female, 235/74 vs. 197/113, age, 58±10 vs. 50±8 yr), using a Multiplexed SNP MassExtended assay (Sequenom MassARRAY RS1000).
Results. rs451360 was associated with a decreased risk of lung cancer (Odds Ratio, 0.59, 95%CI, 0.40-0.87; $P$= 0.007, $\chi^2$ test). Subsequent co-dominant model analysis showed that the genotype "GT" of rs451360 was associated with decreased risk (OR, 0.62, 95%CI, 0.39-0.99; $P$=0.0034). The "CC" genotype of rs402710 was associated with increased risk in males using either a co-dominant model analysis (OR, 2.07, 95%CI, 1.17-3.65; $P$=0.047) or a dominant model analysis (OR, 2.06, 96%CI, 1.17-3.64; $P$=0.021).
Discussion. A protective allele and a gender-specific risk allele in CLPTM1L gene are identified regarding lung cancer susceptibility in the Han Chinese population. Supported by grants from New Zealand-China Scientist Exchange Program and the Ministry of Science and Technology of China.

Timofeeva M N et al (2012) Human Mol Genetics 21:4980-4995

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**Synergistic Regulation of UDP-Glucuronosyltransferase (UGT) 1A8, -1A9 and -1A10 Gene Expression by Caudal-Related Homeodomain Protein 2 (Cdx2) and the Hepatocyte Nuclear Factor 4α (HNF4α)**

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Introduction. UGT1A8, -1A9 and -1A10 are the UGT1A isoforms that are highly expressed in the intestinal tract. Their proximal-1kb-promoter regions are highly similar (>75%) and possess important sequence motifs. Binding elements for the intestine-specific transcription factor Cdx2 and the liver-enriched factor HNF4α have been identified within the UGT1A8, -1A9 and -1A10 proximal-promoters. The role of HNF4α and its interaction with Cdx2 in the intestinal expression of UGTs has not been investigated.
Aims. To determine the role of HNF4α in the regulation of UGT1A8, -1A9 and -1A10 by Cdx2.
Methods. In Vitro study in transiently transfected Caco-2 cells using promoter-luciferase constructs and mutational analysis. RT-qPCR analysis to examine expression of endogenous UGT mRNAs.
Results. We define a novel functional HNF4α site in the proximal promoters of the UGT1A8, -1A9 and -1A10 genes. We also show for the first time that HNF4α and Cdx2 synergistically activate these three UGT promoters in Caco-2 cells. Mutational analysis of the UGT1A8 promoter shows that the novel HNF4α site located at -31 to -44 is essential for the synergy between HNF4α and Cdx2, but does not mediate regulation by HNF4α alone. Further analysis shows that the synergy does not involve a previously defined HNF4α motif located at -808 to -821. Consistent with the UGT1A8 promoter analysis result, the endogenous UGT1A8 mRNA is induced by Cdx2 and synergistically by Cdx2 and HNF4α. UGT1A9 and -1A10 mRNA levels show increase in response to both Cdx2 and HNF4α, but not a synergistic response to the combination of Cdx2 and HNF4α.
Discussion. These findings provide a novel function for Cdx2 and HNF4α in cooperatively controlling intestinal UGTs regulation. They also show that HNF4α has a profoundly different function in regulation of intestinal Vs hepatic UGT expression. The synergistic mechanism described here may be critical for differentiation-dependent UGT expression in intestine.
Gregory P. A., et al (2004) Mol Pharmacol 65:953-963