ePoster abstracts

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Senescence of cardiac and perirenal adipose tissue is increased by obesity and diabetes in mice.

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Introduction. The prevalence of heart failure (HF) is increasing, particularly in the aged and diabetic populations. Recent studies have suggested a potential link between a cellular ageing process, known as senescence, and the development of diabetes. It is however, not completely understood whether the senescence also plays a role in the associated development of HF.
Aims. To determine whether there is evidence of cellular senescence in heart and adipose tissues of aged mice with and without obesity and diabetes.
Methods. Male FVB/N mice commenced a high fat diet (HFD) from 6 (58-week HFD) weeks of age and all mice received streptozotocin (55 mg/kg i.p.) or citrate vehicle for 3 days at 26 weeks of age. At 64 weeks of age (study endpoint) left ventricle and perirenal adipose tissue was collected and snap frozen for gene expression and protein abundance of markers of senescence (p16, p21, p53), oxidative stress (NADPH oxidase subunit, NOX2; superoxide dismutase, SOD-1 isoform) and inflammation (IL-1β, IL-6, ICAM-1).
Results. Mice that received HFD from 6 weeks of age had a significant increase in body weight when compared to the chow control groups. Blood glucose levels were also increased in the STZ groups for both the chow and HFD groups indicating the development of diabetes. The 58-week HFD group had an increase in cellular senescence markers p53 in perirenal fat and p16 in both the LV and perirenal fat. There was also a significant increase in oxidative stress levels in the perirenal fat group with an increase in NOX2 accompanied by a decrease in endogenous antioxidant SOD1 expression in the 58-week HFD group when compared to the chow-citrate control. These significant changes were not seen in the LV samples. Both the LV and perirenal fat samples also saw a significant increase in the fibrosis marker connective tissue growth factor (CTGF) in the 58w HFD group.
Discussion. This study reveals increased cardiac and adipose cell senescence in aged, obese mice with evidence that diabetes causes an additive effect. The senescence is associated with increased oxidative stress. Our findings suggest that senescence is more marked in perirenal fat compared to cardiac tissue but this requires require further investigation.

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The effect of human amnion epithelial cells in an experimental model of chronic stroke

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Introduction. Stroke is a leading cause of mortality and morbidity with up to 50% of survivors become chronically disabled. Currently available treatments such as tissue plasminogen activator and endovascular thrombectomy both have narrow therapeutic windows with limited patient eligibility. Multiple injury mechanisms are activated following stroke onset, resulting in prolonged brain inflammation, apoptosis and ongoing clinical deficits. Previously, we have demonstrated that treatment with human amnion epithelial cells (hAECs) is neuroprotective when administered within 60-90 minutes following stroke. However, approaches to improve outcomes in chronic stroke have not been extensively investigated and there is currently no standard pharmacological intervention available.
Aim. To examine the effect of hAECs when administered after 2 weeks of stroke in aged mice.
Methods. C57BL/6 male (n=28) and female (n=30) mice (aged 10-14 months old) were subjected to photothrombotic stroke directed to the left M1 cortex (n=21) or sham surgery (n=37). At weeks 2 and 5 post-stroke, mice were treated intravenously with either vehicle (saline, n=28) or $1x10^6$ hAECs (n=30). Cylinder tests were performed to assess motor function prior to stroke, as well as at weeks 2, 5 and 8 post-stroke. Mice were euthanised at 8 weeks post-stroke for tissue analysis. Data were expressed as mean±SEM and analysed by mixed model analysis using GraphPad Prism.
Results. When rearing, stroke-operated mice expressed forepaw asymmetry in that they favoured the use of their unaffected (left) forepaw at all times post-stroke. Motor impairment was found to be similar in mice treated with vehicle or hAECs at 5 weeks (left:right asymmetry = veh:328%; hAEC:297%, P=0.82, unpaired t-test) or 8 weeks (veh:237%; hAEC:338%, P=0.31, unpaired t-test).
Conclusion. Our data indicate that when administration of hAECs is delayed for 2 weeks after stroke, there is no long-term benefit for motor impairment. Further studies are needed to understand the full therapeutic time window for intravenous hAECs following stroke.

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Benefit vs harm: how do statins stack up?

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Introduction. In chronic or persistent disease, the relative benefit and harms of long term drug therapy for an individual patient are often unclear. Significant symptoms, the wish to improve quality of life, effective drug marketing and clinician enthusiasm can drive polypharmacy and increased dose, which increase adverse effects (AEs). The number of patients who need to be treated to prevent one clinical event (i.e. benefit, NNTB) can be weighed against the number for one patient to experience one harmful AE (NNTH), perhaps more meaningfully estimated for 1 rather than 5 years from the patient perspective. Depending on AEs, and the specific physiological and pharmacological factors in each patient, the risk-benefit, expressed as a ratio of NNTB:H, is a relevant metric.

Aims. To establish the NNTs for statins, spironolactone and aspirin in preventative treatment.

Methods. Benefit-harm on chronic drug treatment was estimated by NNT, 1 / Absolute Risk Reduction, ARR (Control Event Rate – Treatment Event Rate, %, *per annum*), in largest long term clinical trials.

Results.	**NNTH**	**NNTB**	(*per annum*)
		Any CV event	***Total mortality***
Statins			
WOSCOPS (pravastatin, 1° prevent^{n})	21 (7-70)	204	558 (NS)
HPS (simvastatin, 2° prevent^{n})	21 (7-70)	56	287 (p=0.0003)
Spironolactone (HF)	11	21	16
Aspirin (secondary)	106 (bleeding)	56	143

Discussion. Harms are less tolerated or acceptable in preventative treatment. Although the above estimates are based on selected patients in the clinical trials, potentially different to many seen in practice, our analysis suggests that NNTB:H in secondary prevention appears typically, at best, 2-4 (presuming aspirin has more AEs than just bleeding). Importantly, the limited impact of statins on total mortality, which summates efficacy and harms, suggests that AEs may have consequences.

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A systematic review of adverse drug reactions or adverse drug events of heart failure treatment in frail older adults

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Introduction. Frail older adults with heart failure (HF) have a high risk of mortality and morbidity and may be more vulnerable to adverse outcomes due to multiple co-morbidities and polypharmacy.

Aims. To determine the type, prevalence, causality and severity of adverse drug reactions (ADRs) or adverse drug events (ADEs) reported in frail older adults related to their HF treatment.

Methods. A systematic search of electronic databases (e.g. CENTRAL, MEDLINE, Embase, Ageline, CINAHL, International Pharmaceutical Abstracts, PsychInfo, Scopus), registries and citations was conducted according to PRISMA 2020 checklist. Eligible studies included randomised controlled trials (RCTs) or observational studies of people diagnosed with HF, aged $\geq$65 years, with frailty defined by an objective and validated measure, and reported ADRs or ADEs of guideline directed HF treatments. Two reviewers screened all studies (Kappa=0.88) and assessed the quality (GRADE) and risk of bias (RoB2, ROBINS-I). A second reviewer validated data extracted from 20% of studies.

Results. On preliminary analysis, two RCTs (n=15,032) and three cohort studies (n=1644) were included. Severe ADEs such as mortality and hospitalizations were higher in frail compared to non-frail patients (HR 1.18-2.19, P<0.001) across all HF treatments reported in the RCTs. One observational study reported ADRs according to the Naranjo and Hallas scales. Fourteen percent (n=13/96) of early readmissions were attributed to ADRs and two were attributed to digoxin use. Falls, tiredness and nausea were classified as 'probably avoidable' ADRs, as alternative options or mitigating treatments existed. Analysis of ADEs in a RCT showed that compared to robust patients, very frail patients (frailty index score$\geq$0.311) taking sacubitril/valsartan, aliskiren or ACEIs/ARBs were twice as likely to fall and more likely to experience hypotension, hyperkalaemia, fractures, angioedema and drug discontinuation (P<0.001).

Discussion. Frail older adults were more at risk of severe ADRs or ADEs. ADRs or ADEs were not routinely reported in well-defined frail populations, demonstrating a need for investigation and inclusion in clinical trials and future research.

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Effect of theTRPC3/6 activator GSK1702934A on rodent isolated mesenteric arteries

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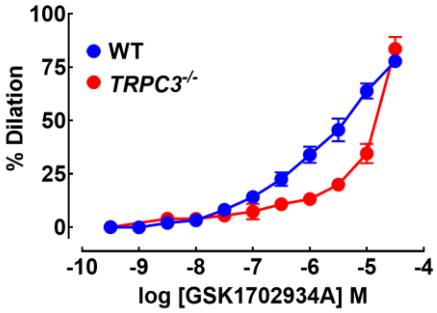
Introduction. Endothelium-dependent dilation (EDD) of arteries is mediated by Ca^{2+}-dependent mechanisms, yet Ca^{2+}-signalling in vascular endothelial cells is poorly understood. Transient receptor potential (TRP) channels may be involved (Senadheera *et al.*, 2012).

Aims. This study investigated the effects of the TRP canonical-types 3 and 6 (TRPC3/6) channel activator GSK1702934A and other vasodilators on EDD in arteries from rats and TRPC3-knockout mice.

Methods. Wild-type or *TRPC3*$^{-/-}$ male mice or Sprague-Dawley rats were anesthetized (isoflurane inhalation or sodium thiopentone 100 mg/kg i.p., respectively) and euthanized. Small mesenteric arteries were isolated, placed in a pressure myograph at 60 mmHg and pre-constricted with phenylephrine (1 µM).



Results. GSK1702934A caused concentration-dependent dilation of vessels from wild-type mice, the response to 1 and 3 µM was reduced by 60% in arteries from *TRPC3*$^{-/-}$ mice. (Figure). Nitric oxide (NO)-dependent EDD induced by acetylcholine (ACh) was greatly reduced in arteries from *TRPC3*$^{-/-}$ mice, but dilation caused by the NO-donor sodium nitroprusside was enhanced by *TRPC3* deletion. GSK1702934A also caused concentration-dependent dilation of rat mesenteric arteries that was greatly reduced by the NO synthase inhibitor L-NAME (100 µM). ACh-induced dilation of these vessels was inhibited by L-NAME and the TRPC3 inhibitor Pyr10, the effects of these drugs were not additive.

Discussion. GSK1702934A induced dilation of murine mesenteric arteries partially dependent on TRPC3. TRPC3-mediated Ca^{2+} entry into vascular endothelial cells may be vital for receptor-stimulated NO accumulation

Senadheera S *et al.* (2012) Cardiovasc. Res. 95:439-47.

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Cardiac recovery from ischaemia and mitochondrial responses with inhibition of trimethylamine-N-oxide in a murine model of pre-diabetes

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Introduction. Trimethylamine-N-oxide (TMAO) is a gut metabolite that has received attention for its putative involvement in cardiovascular disease (CVD). Though whether its elevations are causative or merely a biomarker of CVD remains unknown. Elevations are observed in diabetes, a strong promoter of CVD, both of which are characterised by mitochondrial dysfunction. Studies performed on myocardial responses to TMAO are few, less so in the context of diabetes. Thus, we wish to address the gap.

Aims. The purpose of this study was to investigate the effects of inhibiting TMAO production in a mouse model of pre-Type 2 diabetes (Db) on cardiac mitochondrial function, and functional recovery from Ischaemia/Reperfusion (IR).

Methods. T2D was induced with a single injection of streptozotocin (75 mg/kg) followed by 14 weeks of high-fat, high-sugar feeding with or without 3,3-dimethyl-1-butanol (DMB), an inhibitor of TMAO synthesis, in drinking water (1.0% v/v) in 10-wk old male C57Bl/6 mice. Metabolic assessment included body weight charting (weekly), glucose tolerance tests (GTTs) and fasting blood samples (performed at weeks 6 and 12). Baseline and post I/R contractile function and left ventricular mitochondrial function was assessed on the Langendorff apparatus and a Oroboros Oyxgraph-2k instrument (using the SUIT1 protocol) respectively.

Results. T2D mice were significantly heavier than the control group at the end of the study. Mice in the DMB groups weighed less throughout the study. T2D mice had significantly higher fasting glucose and GTT results, whilst DMB alone had no effect in either control or T2D groups at weeks 6 and 12 of the study. Mitochondrial data at baseline indicate that DMB lowers CI&II linked respiration and maximum respiratory capacity (MRC), in control mice whilst T2D alone and combined with DMB increased CI and CI&CII linked respiration, and MRC. Preliminary data (not yet complete) show no change in recovery from I/R.

Discussion. While inhibiting TMAO doesn't seem to affect glucose handling or recovery from I/R, its inhibition appears to induce changes in mitochondrial function.

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Developing a class of cardiac ryanodine receptor stabilising antiarrhythmics

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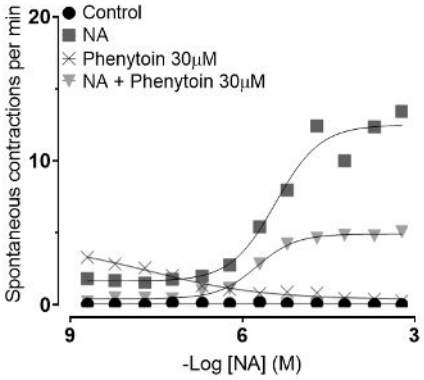
Introduction. Phenytoin, a hydantoin derivative used as an anti-convulsant, is known to exhibit weak Na^+ channel blockade in cardiomyocytes. Recent studies uncovered that phenytoin functions to inhibit diastolic Ca^{2+} leak from cardiac ryanodine receptor (RyR2) channels of human failing hearts without adversely inhibiting Ca^{2+} release during systole or impacting normal Ca^{2+} RyR2 mediated release from healthy hearts (Ashna et al., 2020).
Aims. Develop a novel antiarrhythmic to restore normal Ca^{2+} release from RyR2 from failing hearts utilising phenytoin as a lead compound.
Methods. The effects of phenytoin were observed in 46 non-failing human right atrial appendages (RAA) and 7 explanted ventricles with advanced heart failure. Effects on inotropy and antiarrhythmic properties were examined at concentrations including 10, 30 and 100 µM.



Results. In RAA, phenytoin at concentrations of 10 - 30 µM did not reduce the potency or maximal effect of NA at the β_1-adrenoceptor. Phenytoin significantly inhibited spontaneous beats arising during functional arrhythmia protocols in RAA. In human failing ventricular trabeculae, 30 µM phenytoin, reduced spontaneous beats stimulated through NA.
Discussion. We conclude that phenytoin, with known RyR2 stabilising capabilities, significantly reduces spontaneous beats in both trabeculae from RAA and ventricle from human non-failing and failing hearts respectively. Phenytoin at concentrations below 30 µM does not affect contractile strength. Further studies are required to prove mechanism of action.
Ashna, A., et al. (2020). Mol. Pharmacol. 97:250-258

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Characterising a new and improved model of diet-induced metabolic syndrome

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Introduction. Metabolic syndrome (MetS) is a complex multifactorial disease and one of the risk factors for cardiovascular disease. Importantly, perivascular adipose tissue (PVAT) is continuous with the adventitia of blood vessels and a recognized regulator of vascular function. PVAT expansion in MetS promotes inflammation and vascular dysfunction, but the sexual dimorphisms of this are poorly understood due to unreliable animal models. Aims. Using a new mouse model of diet-induced metabolic syndrome developed in our laboratory, we characterised the aortic PVAT immune cell profile and determined the effect of PVAT vascular function in males and females. Methods. 6-week-old male and female C57BL/6 mice were fed either a high-fat diet (43% kcal in food) with high sugar and salt in their drinking water (10% high fructose corn syrup and 0.9% NaCl; HFSS), or normal chow diet (NCD) for 10 weeks. Physiological parameters were measured weekly and fortnightly. At end point, blood and aortic immune cell populations were characterised using flow cytometry. Endothelium-dependent relaxation to acetylcholine (ACh) in mouse abdominal aorta rings (with and without PVAT) was measured using wire myography. Results. Compared to NCD, male and female HFSS mice exhibited increased cumulative weight gain, fasting blood glucose and systolic blood pressure ($P<0.05$, n=34-41), all of which are clinical characteristics of MetS. In males, HFSS diet significantly increased ($P<0.05$ vs. HFSS female) aortic pro-inflammatory monocytes (Ly6CHi+) and neutrophils (Ly6G+). With increases in male ($P<0.05$ vs. HFSS) systemic myeloid-derived cells (CD11b+), neutrophils (Ly6G+) and B cells (B220+). Females were protected from HFSS-induced systemic inflammation. The presence of PVAT blunted endothelium-dependent relaxation in HFSS males ($P<0.05$ vs. NCD male) but enhanced endothelium-dependent relaxation in HFSS females. Discussion. We developed a clinically relevant HFSS diet-induced model of MetS and despite metabolic disturbances being present in both sexes, systemic inflammation was only observed in males. Moreover, both males and females show changes in vascular function with and without PVAT, but with contrasting effects in MetS. These data highlight the sexual dimorphisms in PVAT and support the importance of PVAT as a major regulator of vascular health.

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Sex and age alter cardiometabolic response following Polypharmacy in mice

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Introduction. Cardiovascular and metabolic diseases are common in old age, with increasing proportions of individuals prescribed polypharmacy (concurrent use of ≥ 5 medications). Polypharmacy and high Drug Burden Index (DBI – cumulative exposure to anticholinergic/sedative drugs) are known to impair function in older adults and mice. Preclinical models can provide a mechanistic understanding of these exposures on organ function.
Aims. Using C57BL/6 mice, we aimed to assess the effects of polypharmacy with high DBI (HDBI) on several cardiometabolic features in mice of different ages and sexes.
Methods. Young (4 months) and old (23 months) mice of both sexes, were randomised to receive control or HDBI polypharmacy feed (simvastatin, metoprolol, oxybutynin, oxycodone, and citalopram; at therapeutic doses) for 4 weeks. BP, fasting glucose and insulin levels were measured pre- and post-treatment. Echocardiography (echo) was performed at the end of the study, left ventricles (LV) and livers were collected for analysis.
Results. After treatment, HDBI decreased heart rate and cardiac output in all animals ($p<0.05$), but only reducing systolic/diastolic BP in young mice and significantly increasing systolic BP in old females. In control groups, LV chamber size was significantly larger in old than in young mice of the same sex. HDBI decreased LV chamber size and increased wall thickness, ejection fraction and fractional shortening, relative to control. The effects of HDBI on these echo measures were greater in males than females, indicating treatment*sex interactions in HDBI groups ($p<0.05$). Fibrosis analysis showed that HDBI significantly increased LV collagen content, compared to control in females but not males. For metabolic measures, HDBI decreased insulin sensitivity in all mice, relative to the corresponding control. HDBI also decreased the hepatic gene expression of insulin receptor and insulin receptor substrate-1 in young and old males.
Discussion. Our results suggest that HDBI polypharmacy may affect the cardiometabolic profile differently according to age and sex. Future studies are required to further elucidate the pathogenesis of these observations.

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Pharmacological Inhibition of Interleukin-18 Prevents DOCA/salt-induced Hypertension and Renal Inflammation

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Introduction. Circulating levels of the pro-inflammatory cytokine, interleukin-18 (IL-18), are elevated in patients with hypertension and chronic kidney disease (CKD)[1]. Moreover, genetic deficiency of IL-18 prevents the development of high blood pressure and renal injury in preclinical models of these diseases[2].
Aims. To determine if pharmacological inhibition of IL-18 is similarly protective against high blood pressure, renal inflammation and damage in deoxycorticosterone and high salt (DOCA/salt)-induced hypertension and CKD.
Methods. Male C57BL/6J Mice were randomly assigned to receive a control IgG or anti-IL-18 neutralising monoclonal antibody (mAb; 30 mg/kg, i.p. bolus every 3 days). Three days after the first injection, mice were uninephrectomised and treated for a further 21 days with either DOCA (2.4 mg/d, s.c. pellet) plus high salt (0.9% in drinking water), or a placebo pellet (s.c.) plus normal drinking water. Blood pressure (BP) was measured weekly (tail-cuff), while renal function was measured at baseline and at endpoint by transdermal glomerular filtration rate (tGFR) detection. Flow cytometry was used to determine renal immune cell infiltration.
Results. Baseline systolic BPs were not different between mice treated with the anti-IL-18 mAb (118 ± 2 mmHg) versus the control IgG (122 ± 3 mmHg). However, anti-IL-18 mAb treatment blunted the hypertensive response to DOCA/salt, with systolic BPs only reaching 139 ± 6 mmHg cf. 159 ± 6 mmHg in control IgG-treated animals. Anti-IL-18 mAb treatment also reduced 1K/DOCA/salt-induced leukocyte (CD45+) accumulation in the kidneys by ~50% compared to control IgG treatment, with further analysis of leukocyte subsets revealing fewer T cells (CD3+) and myeloid lineage cells (CD11b+). Finally, kidney function declined by ~30% over the 21-day DOCA/salt treatment in control IgG-treated mice. The decline in kidney function appeared to be less severe (~17% reduction) in anti-IL-18-treated mice (n=7).
Discussion. IL-18 neutralisation affords protection against 1K/DOCA/salt-induced hypertension and renal inflammation. Thus. IL-18 may represent a potential therapeutic target to treat CKD.

[1]Formanowicz D (2015) *Sci Rep*, 5:18332
[2]Thomas et al (2021), *Hypertension. In press*

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Re-evaluating plasma for tacrolimus therapeutic drug monitoring: relationship between trough blood and plasma tacrolimus concentrations

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Introduction: The immunosuppressant tacrolimus (Tac) has a narrow therapeutic window and highly variable intra- and inter-individual pharmacokinetics warranting therapeutic drug monitoring (TDM)[1]. In renal transplants TDM allows dose individualisation by maintaining blood trough Tac concentrations (C_0) within 4-12 ng/mL[1]. High binding within erythrocytes and to plasma proteins[2] makes interpreting blood C_0 difficult due to large variability in binding early post-transplantation. Alterations in haematocrit cause variability in blood C_0 that may not reflect changes in unbound C_0 and may lead to misguided dose adjustments and adverse clinical outcomes. Measuring Tac in plasma may overcome the confounding effect of haematocrit. Aims: Investigate the relationship between blood and plasma Tac concentrations and the effect of clinical covariates. Methods: Blood C_0, biochemistry and demographics collected from routine TDM records 0-60 days post-surgery in de novo renal transplant recipients. Blood and plasma C_0 measured using LC-MS/MS. The effect of clinical covariates on the plasma:blood (P:B) ratio investigated using Spearman rank correlations and Mann-Whitney U test. Results: In 167 samples from 21 transplant recipients median (range) blood and plasma C_0 were 7.6 ng/mL (2.0 – 21.4 ng/mL) and 746 ng/L (165 – 2620 ng/L) respectively, with P:B ratio 0.10 (0.049 – 0.357). The correlation between blood and plasma C_0 (r_S = 0.596, P = <0.0001) explained 35% of variability. The P:B ratio correlated with haematocrit (r_S = 0.292, P <0.0001) but not with time post-transplant, total protein, serum albumin, sex or age. Discussion: Haematocrit poorly predicted (8.5%) variability in the P:B ratio suggesting that correcting for haematocrit may not adequately predict plasma tac C_0. Plasma may serve as a better matrix for Tac TDM and plasma Tac C_0 may better reflect unbound C_0[3] and may better predict clinical outcomes.

[1]Brunet M et al (2019) Ther Drug Monit 41:261-307. [2]Zahir et al (2004) Br J Clin Pharmacol 57:298-309.
[3]Sikma MA et al (2020) Clin Pharmacokinet 59:771-780

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Preliminary validation of a LC-MS/MS method to quantify dolutegravir in human plasma.

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Introduction. HIV/AIDS is a major health problem in many developing countries including Papua New Guinea (PNG). Currently, the main drug therapy for HIV/AIDS in PNG is dolutegravir (DTG) -based regimen at a once-daily fixed dose of 50 mg (in combination with tenofovir disoproxil fumarate and lamivudine). DTG was recently recommended by WHO (2019), and besides clinical trials, pharmacokinetic studies are scarce and its potential toxicity was not entirely elucidated.
Aims. Develop a LCMS/MS method to quantify plasma DTG to support clinical pharmacology research in PNG HIV/AIDS patients.
Methods. DTG was extracted from plasma (100 µL) by protein precipitation with acetonitrile and 1 µL resolved on a C18 column (Acquity Premier HSS T3 1.8 µm, 2.1 x 150 mm, 40°C). Mobile phase used was HPLC grade water (A) and acetonitrile (B) with 0.1% formic acid, with 5.5 min gradient (start 50%B, increasing up to 90%B at 2.5 min, hold 1 min and back to the start condition). Detection was by a LCMS8040 triple quadrupole MS (Shimadzu). Calibration curves ranged from 8.75 to 8400 ng/mL. The parameters tested were inter-day (n=3) and intra-day (n=3) precision, accuracy, sensitivity, recovery, specificity, linearity and matrix effect (ME).
Results. Intra- and inter-day imprecision (CV) for quality control samples and lowest calibrator ranged from 2.6-3.1% and 1.6-5.1%, respectively. Mean accuracy ranged from 99.5-103.3%. Calibration curves were linear with weighting factor of $1/x^2$ and r^2 ranging from 0.991 to 0.999. No interfering peaks were observed in the 7 blank plasmas tested for specificity. Recovery and ME were assayed with low and high quality control samples, ranging from 87- 89% and 5-19%, respectively.
Discussion. Preliminary tests demonstrate a precise and specific method to quantify DTG in human plasma by LC-MS/MS, which will be applied in a future study. However, further assay development and full validation is required, including stability under different conditions.

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Safety and tolerability of *Annona muricata* leaf product in people living with cancer: research protocol for an open-labelled pilot study

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Introduction. *Annona muricata*, also known as graviola, soursop and guanabana, is a herbal product that is widely used as part of self-care by people living with cancer. Anecdotal clinical evidence suggests this herb has potential anti-cancer activity. There are numerous pre-clinical studies detailing *Annona muricata* main bioactive constituents attributed to anti-cancer and chemoprotective properties (Chan et al, 2020). To date, clinical studies evaluating the efficacy, safety and tolerability of *Annona muricata* in people living with cancer are limited.
Aims. The aim of this clinical study is to investigate the safety and tolerability of a commercially available *Annona muricata* leaf product in people living with advanced malignancy.
Methods. This is an open label pilot clinical study investigating the safety and tolerability of *Annona muricata* leaf product in people with stage III and IV cancers of any type, who are not undergoing chemotherapeutic treatment.
Results. Participants (n = 24) will be allocated to one of four treatment groups for 12 weeks. Each group will commence at different timepoints and be assigned a specific daily dose of either 375 mg, 750 mg, 1500 mg or 3000 mg of *Annona muricata* using a herbal product of known quality. Data collection will be conducted at baseline, weeks 3, 6, 9 and 12. The primary outcome of this study is to report measures for safety and tolerability of *Annona muricata* leaf including any adverse effects experienced and the proportion of participant completing the study. The secondary outcome is to report biomarkers of inflammation (CD3, CD4, CD8, CD16/56, CD19 and lymphocyte helper: suppressor ratio), cancer specific quality of life scores as measured by Functional Assessment of Cancer Therapy – General questionnaires and Functional Assessment of Chronic Illness Therapy – Fatigue questionnaires, disease status (standard care) and survival.
Discussion. The results of this study will inform further research and provide evidence-based data to support clinicians involved in the care of people living with advance malignancy who choose to use *Annona muricata* leaf product.

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Disease duration and remission in rheumatoid arthritis patients treated with tocilizumab and/or conventional synthetic disease modifying anti-rheumatic drugs: results from 5 phase III randomized clinical trials

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Introduction. The underlying pathophysiology of rheumatoid arthritis (RA) changes early in the disease and patient response to treatment may decrease over time. The interval in which patients are more likely to response is not defined yet. Disease duration has been previously investigated as a potential treatment response predictor with contradictory results. Therefore, the association between RA duration with outcomes requires clarification.
Aims. To investigate the association between RA duration at baseline and remission in RA patients treated with disease-modifying antirheumatic drugs (DMARDs).
Methods. Data were pooled from 5 RCTs of RA. The primary outcome was time to first remission according to the simplified disease activity index (SDAI). Available data included baseline BMI, age, RA duration, number of previous DMARDs, race, sex, seropositivity and baseline SDAI. RA duration was initially modelled as a continuous variable. Potential non-linear associations were evaluated using restricted cubic splines and visual checks. The association between RA duration categories and remission was also assessed to facilitate clinical implementation. Disease duration was categorized into early (≤1), intermediate (1-2, 2-5) and established (>5; 5-10, 10-15 and >15) disease. The association between baseline RA duration and remission was assessed via Cox proportional hazard analysis.
Results. Analysis included data from 5502 RA patients. As continuous variable, increased disease duration was associated with less frequent remission (P<0.001). 1490 (27%) patients were classified as early RA, 1522 (28%) were intermediate and 2478 (45%) were established RA. Early RA patients were more likely to achieve remission compared with patients who had >15 years duration on univariable (HR 1.61 (95%CI 1.29-2.00, P<0.001)) and adjusted (HR 1.54 (95%CI 1.22-1.94, P=0.002)) analyses. There was no difference in remission rates between patients with disease duration of 5-10 (HR1.05(95%CI (0.83-1.23) or 10-15 (HR1.037(95%CI 0.83-1.24)compared to the >15 years group.
Discussion. Patients with disease duration ≤1 year were more likely to achieve remission. This study also suggests that RA duration between ≤1 and ≤5 is the interval in which RA patents are more likely to achieve remission.

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A new multidomain frailty index and the hospital frailty risk score: tools for pharmacoepidemiology in older inpatients

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Introduction. Frailty, a measure of a person's vulnerability to external stressors, is an important determinant of health care needs, medication use and outcomes for people in hospital, at the individual and system levels.
Aims. To compare a new multidomain frailty index (electronic frailty index–acute hospital; eFI-AH) with the hospital frailty risk score (HFRS) with respect to (1) distribution; (2) agreement; (3) association with age; (4) association with anticholinergic and sedative medication use measured with the Drug Burden Index (DBI); and (5) prediction of health care utilisation and outcomes, amongst older inpatients.
Methods. The eFI-AH and HFRS were calculated for people aged 75 years or older admitted to the Royal North Shore Hospital between October 1 2019 and September 30 2020 (n=6,771). The prevalence of frailty measured by eFI-AH and HFRS, degree of agreement in frailty ratings between the tools and the relationship of frailty with age and with DBI (in the subgroup receiving care from geriatric medicine, n=1162) were evaluated. Predictive ability of the tools with the following 4 outcomes was evaluated: in-hospital mortality, long hospital stay (>10 days), unplanned all-cause readmission and fall-related readmission within 28 days. The discriminative ability was assessed using the area under the receiver operating characteristic curve (ROC-AUC) and area under the precision recall curve (PR-AUC).
Results. The eFI-AH had a median of 0.17 (IQR 0.11–0.26) while the median HFRS was 3.2 (IQR 0.4–7.7). Moderate agreement was shown between the tools (kappa 0.42 [95% CI 0.40–0.44], Pearson 0.61 [0.59–0.62]). The average eFI-AH increase per year was steeper than the average HFRS increase (0.6%, 0.2% respectively). Both eFI-AH and HFRS models for predicting long hospital stay showed good discrimination (AUC-ROC 0.75, 0.81, PR-AUC 0.39, 0.51, respectively) and calibration (slope 1.054, 0.992, intercept -0.010, 0.002 respectively). Preliminary results showed DBI was associated with eFI-AH but not with HFRS (adjusted odds ratio 1.16 [1.09-1.24], 0.99 [0.88-1.11], respectively).
Discussion. This study confirmed that eFI-AH and HFRS identify different cohorts of acute inpatients as frail. Only eFI-AH was associated with DBI. Both identify inpatients at increased risk of adverse health outcomes, although the discriminative ability suggested that the tools would be more relevant at a system level than at the individual level.

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National roll-out: The Goal-directed Medication review Electronic Decision Support System (G-MEDSS)© in practice

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Introduction. The Goal-directed Medication review Electronic Decision Support System (G-MEDSS)© provides guidance for healthcare practitioners conducting medication reviews, to tailor care to meet their patients' goals and preferences. G-MEDSS consists of The Goals of Care Management Tool (GCMT), The Drug Burden Index (DBI) Calculator© and the revised Patients' Attitudes Towards Deprescribing (rPATD) questionnaire.
Aims. This study aimed to describe the 1) users of G-MEDSS; 2) clinical settings where G-MEDSS was used; and 3) patients for whom G-MEDSS was used; during a national implementation study.
Methods. Prospective cross-sectional evaluation (1st May 2020 – 31st May 2021). The study was advertised to registered medical practitioners and pharmacists through relevant professional organisations. Participants were invited to register to use G-MEDSS within their clinical practice settings. De-identified data about the users and their patients were collected through the website and descriptively analysed.
Results. A total of 129 participants (115 pharmacists and 14 medical practitioners) registered to use G-MEDSS, with most participants from NSW (n=35, 27%). These participants used G-MEDSS for 95 patients (mean age(SD) 76.8 (11.3)), predominately during medication reviews in the home (n=60,63%) and residential care (n=27,28%). Participants used the GCMT, DBI Calculator and rPATD for n=28 (29%), n=90 (95%), and n=23 (24%) of patients, respectively. The most common goal reported by patients was "optimising quality of life" (n=23, 36.5%). The mean(SD) DBI score for patients was 1.41(1.1) and the mean(SD) number of medications per patient was 10.0(4.1). The 793 medication recommendations made by clinicians who used G-MEDSS consisted of 413 (52%) for no change, 190 (24%) to continue the medication as clinically indicated, 181 (23%) to deprescribe the medication, and 9 (1%) to increase the dose. The proportion of patients who said that they would be willing to have a medication deprescribed if their doctor recommended it was 82.6% (n=19).
Discussion. G-MEDSS is being used within clinical practice primarily by pharmacists to support medication review in the home. Further qualitative studies will determine the barriers and enablers to wider use.

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Polypharmacy and monotherapy treatments exacerbate frailty in mice measured using different assessment tools

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Introduction. Polypharmacy (≥5 medications) and the Drug Burden Index (DBI; measure of cumulative exposure to anticholinergics and sedatives) are associated with increased risk of frailty in observational studies but the pathophysiology of the association is unclear.
Aims. To evaluate the effects of different chronic polypharmacy and monotherapy regimens from middle to old age on the Clinical Frailty Index and Frailty Phenotype, assessed as score and odds ratio, where higher score denotes greater frailty and higher ratio denotes greater odds of being frail, respectively.
Methods. In a longitudinal study, middle-aged (12 months) male C57BL/6J(B6) mice (n=25-40 per treatment) were chronically administered therapeutic doses of medications as monotherapy or in polypharmacy combinations (with various DBIs; zero (DBI: 0), low (DBI: 0.5) and high (DBI:1.6)) in feed and/or water; or control feed/water. Both frailty assessments were performed every 3 months between 12 and 24 months.
Results. Compared to control, polypharmacy and monotherapy therapeutic drug regimens increased the number of frailty deficits for the Clinical Frailty Index (high DBI polypharmacy, oxycodone, oxybutynin and citalopram, $p<0.05$) and the Frailty phenotype assessment (high DBI polypharmacy, oxybutynin and citalopram, $p<0.05$). The odds of developing frailty also increased for high DBI polypharmacy for the Clinical Frailty Index, and low DBI polypharmacy, high DBI polypharmacy and citalopram monotherapy for the Frailty Phenotype (adjusted odds ratio (aOR) = 3.13; 95% CI 1.01 – 9.66) and frailty phenotype assessment: low DBI polypharmacy (aOR = 4.38, 95% CI 1.40 – 13.74), high DBI polypharmacy (aOR = 3.43; 95% CI 1.12 – 10.50) and citalopram monotherapy (aOR = 4.63; 95% CI 1.39 – 15.54).
Discussion. Polypharmacy and monotherapy regimens with DBI >0 induce frailty, with varying degrees, depending on the frailty assessments and analytic methods used. As similar findings are seen in pharmaco-epidemiologic studies, this model provides an opportunity to understand mechanisms underlying these relationship.

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Quantification of ketamine, norketamine, and hydroxynorketamine by UHPLC-MS/MS in human blood plasma

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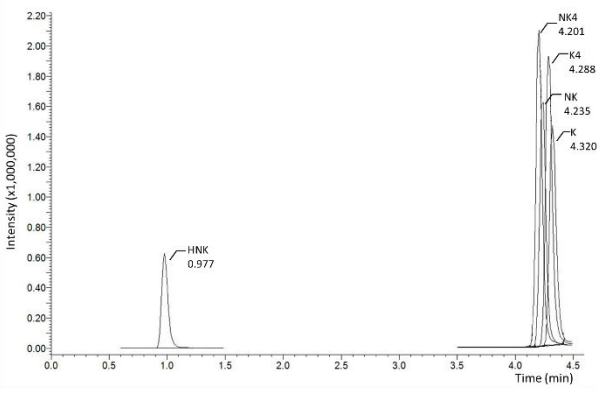

Introduction. Ketamine (KET) is a commonly used anaesthetic drug recently found to possess clinical applications in the treatment of depression. To investigate the metabolisation of KET, we present a fully validated ultra-high performance liquid chromatographic procedure coupled with tandem mass spectrometry (UHPLC-MS/MS) for determination of KET, and its metabolites norketamine (NK), and hydroxynorketamine (HNK) in human blood plasma.
Aims. To develop and validate a sample preparation procedure and UHPLC-MS/MS assay for quantitative determination of KET, NK, and HNK in human blood plasma.
Methods. Concentrations of calibrators containing all 3 analytes prepared based on expected concentrations in range of 0.75-250 ng/ml (KET, NK) and 1.5-500 ng/ml (HNK). Solid phase extraction (SPE) was performed using Strata DE plates (Phenomenex) and elution by gravity. Detection performed on LCMS-8040 and LC separation achieved using Shim-pack XR-ODS III (Shimadzu, Japan).
Results. SPE recoveries for all analytes were above 90%. QC samples intraday precision ranged from 0.9% to 5.5%, inter-day precision ranged from 1.8% to 6.7%. Accuracy ranged from 0.3% to 6.7%. Partial volume analysis precision ranged from 2.8% to 3.4%, accuracy ranged from 0.2% to 2.5%.
Discussion. This assay allows for the extraction and simultaneous analysis of ketamine and 2 of its metabolites in plasma using UHPLC-MS/MS. Validation data for selectivity, recovery, precision, and accuracy were within the required limits. The assay was successfully applied to patient plasma samples, allowing for monitoring of KET concentrations in adult patients receiving KET for treatment of depression. This method can be used in future clinical trials to investigate KET metabolism following therapeutic use, or to determine the extent of its abuse if taken recreationally.

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(C)onsumer focused (E)ducation on p(A)racetamol (S)ide (E)ffects, i(N)adequate (O)utcomes and (W)eaning (CEASE NOW) for individuals with low back pain: Protocol for a feasibility study

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Introduction. Prescription and over the counter medications, such as paracetamol, account for a significant proportion of the direct and indirect costs in managing low back pain. Existing research has not only questioned the efficacy of paracetamol use for musculoskeletal conditions such as low back pain, but also its safety. However, still, the majority of people with low back pain take paracetamol and are unare of the risks. No previous study has investigated the feasibility of a pharmacological education tool for individuals using paracetamol for their low back pain.
Aims. To investigate: (1) the acceptability and experience of participants with the pharmacological education tool, (2) feasibility of recruitment, data collection and outcome measure completion, and (3) participant's willingness to participate in a randomised control trial.
Methods. We will use a single group, repeated measures, mixed methods, feasibility study design. We will recruit a sample of 20 adults with low back pain, using paracetamol, remotely from consumer pain groups. The intervention is a booklet containing education about the safer and more effective alternatives to paracetamol to treat low back pain. Surveys will be completed at baseline and then 1 week and 1 month after receiving the education tool. Semi-structured interviews will also be completed after the study.
Results. Data analysis will be both quantitative and qualitative. Quantitative data will be analysed on variability of means and 95% confidence intervals. Qualitative data will be explored through thematic content analysis. We will judge the study feasible, if, we recruit 20 participants within 3 months of first advertisement, the study design and educational tool are found to be acceptable by the majority of participants, and there is less than 20% missing data for outcome measures, and at least 85% follow up of enrolled participants.
Discussion. The impact of this study could be highly significant, as we could show the potential for consumer education to empower individuals with low back pain to better self-manage their condition, without the need for paracetamol, therefor reducing the burden and cost of low back pain.

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Population pharmacokinetics of intravenous treosulfan in children receiving blood or bone marrow transplantation

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Introduction. Treosulfan is a myeloablative conditioning agent administered to patients prior to blood or bone marrow transplantation for the treatment of haematological malignancies, immunodeficiencies, and genetic diseases. A target range for cumulative treosulfan AUC of 4800 mg*h/L (range 3800 – 6000 mg*h/L) has been identified for patients with immunodeficiencies[1], and may also be indicative for patient cohorts with malignant or genetic diseases.
Aims. To develop a population PK model and explore dose predictions to achieve a target AUC for treosulfan disposition in paediatric patients receiving treosulfan for various haematological conditions.
Methods. 321 concentration time observations (measured by HPLC-UV) were obtained from 47 patients (age 0.22 – 17; median 3.95 yrs) receiving 3 daily doses of treosulfan (30 – 42 g/m^2 cumulative dose) and analysed using nonlinear mixed effects modelling (NONMEM v7.4) software. Treosulfan CL and V were assessed through structural, error, and covariate models. Model evaluation using visual predictive checks (VPC) and bootstrapping assessed model predictability and robustness (n = 1000). A simulated population (n = 10,000) with unique individual characteristics was generated using Monte Carlo simulation and dosing schemes based on patient age, BSA, and model CL were compared.
Results. A one-compartment PK model with inter-individual variability (IIV) on CL and V, and proportional and additive residual error best fit the data. Allometric scaling with exponents of 0.75 and 1.0 described variability in CL and V, respectively. Maturational function using a post-menstrual age covariate influenced CL. Final estimates for CL and V were 16.6 L/h and 40.4 L (IIV 23.6% and 23.7%), respectively. Goodness of fit plots, VPC, and bootstrapping parameters were acceptable. Dosing simulations indicated that model-based dosing was the most reliable dosing protocol vs age- or BSA- based schemes, however AUC was evaluated within the therapeutic range for only 54% of simulated individuals.
Discussion. Therapeutic drug monitoring using a model-based dosing approach as a start point is recommended.
[1]Chiesa R et al (2020) Clin Pharmacol Ther 108(2): p 264-273

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Developing guidelines for the appropriate use of psychotropic medications in people living with dementia and in residential aged care: focus on antipsychotics

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Introduction. The Royal Commission into Aged Care Quality and Safety has highlighted the over-reliance on psychotropic medications in people with dementia and changed behaviours living in residential aged care. A multidisciplinary Guideline Development Group (GDG) are developing clinical practice guidelines for the appropriate use of antipsychotics, benzodiazepines and antidepressants.
Aims. To describe the process for developing evidence-based recommendations related to antipsychotics.
Methods. Clinical questions related to antipsychotics were adapted from existing Australian and international guidelines and prioritised by stakeholders and the GDG. Recent systematic reviews and randomised control trials (RCTs) were identified from systematic database searches. Grading of Recommendations, Assessment, Development and Evaluation (GRADE) was used to rate the certainty of evidence and strength of recommendations. The Evidence to Decision framework was employed to facilitate consideration of benefits and harms, preferences and values, resources, equity, acceptability, and feasibility.
Results. The GDG prioritised three clinical questions related to antipsychotics: risks and benefits of antipsychotics; antipsychotic discontinuation vs. continuation; and interventions to ensure appropriate use. We identified randomised controlled trial (RCT) evidence of varying certainty in relation to first- and second-generation antipsychotics, with a number of evidence gaps related to harms. Literature on the preferences and values of people living with dementia, acceptability, feasibility and potential inequities related to antipsychotic treatment was limited.
Discussion: Evidence from RCTs will be supplemented with evidence from observational studies and used as the basis for producing clinical practice guidelines for clinicians, resources for other healthcare staff, a consumer companion guide and a co-designed implementation and dissemination plan.

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Oral Mucositis, what have we learnt in the last two decades: A narrative review

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Introduction. Oral mucositis (OM) is the most common and inevitable sequela of anti-cancer therapies. OM associated oral pain (OMOP) is reported to be the most burdensome symptom, compromising the patient's functional capabilities, adherence to anti-cancer treatment and psychosocial status; which in turn reduces their overall quality of life (QOL). OM is unavoidable, as such, it is necessary to manage it based on patient symptoms; most notably, oral pain. Topical agents are most preferred in the management of OMOP, compared to their systemic counterparts. At current, there is still limited evidence to support the use of a standard therapy for OM management alone.
Aims. The present narrative review aims to compile the most effective, emerging topical therapies for management of OMOP.
Methods. Databases consisting of Cochrane Database of Systematic Reviews Database, Medline and Embase via Ovid were used to investigate key search terms. Articles retrieved were screened for duplicates, and any articles published prior to 1999 were excluded. A total of 28 articles were included within the final narrative review; 23 of which were Randomised Controlled Trials.
Results. Of the 21 pilot compounds investigated, 8 were found to have a statistically significant role in the management of OMOP. These included topical therapies containing doxepin, ketamine, morphine, indomethacin, diclofenac, benzydamine, rhEGF and phenylbutyrate. Each compound differs in evidence supporting its efficacy according to the malignancy type and location, nature of the treatment received and the initial severity of OM.
Discussion. Heterogeneity across the studies amplifies the need to develop a standardised validated assessment tool, which would allow for accurate comparison regarding the efficacy of therapies in reducing OMOP. Further research is warranted into the pathophysiology of oral pain specifically, as well as to fully elucidate the role of topical therapies in the management of OMOP.

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Optimising adherence to allopurinol: gout patients' perspectives

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Introduction. Gout is the most common form of arthritis in men. Despite effective urate-lowering treatments such as allopurinol, management of gout remains suboptimal. Poor adherence to allopurinol is a key reason for suboptimal gout management, with research suggesting adherence to allopurinol is one of the lowest of any chronic condition.
Aims. To understand the opinions of patients with gout on the factors contributing to poor adherence to allopurinol, and their perspective on strategies, including technological interventions, to support adherence to allopurinol.
Methods. Semi-structured interviews with gout patients currently or previously taking allopurinol were conducted. Questions focused on participants' experiences taking allopurinol, factors affecting their allopurinol adherence, and their opinions on strategies to support their allopurinol medication taking. Interviews were transcribed verbatim and inductive thematic analysis was independently conducted by two researchers to identify emerging themes.
Results. Preliminary findings demonstrated that participants reported both intentional and non-intentional non-adherence to allopurinol. Forgetfulness, negative attitudes towards medication and limited feedback regarding the effectiveness of allopurinol were barriers to adherence. Having a regular medication-taking routine, motivation through the frequency of gout flares, and understanding gout and its treatments were facilitators of adherence. Participants identified the ability to self-monitor urate concentrations, gout management apps and medication reminders as helpful strategies to support them to take their allopurinol regularly.
Discussion. Forgetfulness, negative attitude towards medicines and lack of mechanisms of monitoring treatment response are key barriers to optimising adherence to allopurinol. Ability to self-monitor urate concentrations and digital platforms are potential strategies to overcome these barriers.

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Formative evaluation of the Pharmacist-led INtervention for people with gout (PIN-gout)

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Introduction. The role of pharmacists in assisting general practitioners (GPs) with patient education and medication reviews has been increasingly recognised. Involving pharmacists could enhance the management of gout in primary care and improve the current, poor adherence to urate-lowering therapy (ULT). Optimal management of gout involves self-management support, serum urate monitoring, a treat-to-target (T2T) urate strategy, and acute flare prevention. Incorporating these elements of care, we are developing a pharmacist-led intervention for better management of gout.
Aims. To conduct a formative evaluation of the proposed pharmacist-led intervention in primary care to inform its further development and to examine the acceptability and feasibility of its implementation through GP perspectives.
Methods. We conducted semi-structured interviews with GPs in Australia and used the Consolidated Framework for Implementation Research (CFIR) to guide interview questions and data analysis across five domains of the CFIR.
Results. Prevailing perspectives from interviews with 11 GPs indicate that the intervention is deemed feasible, adaptable and beneficial to both GPs and patients in managing gout (Intervention characteristics), where poor ULT adherence and the need for patient education and self-management support tools are recognised (Outer setting). However, some GPs expressed reservations about pharmacists preparing T2T urate plans, despite their existing collaborations with pharmacists in routine care, and thus recommended direct communication between GPs and rheumatologists (Inner setting). Whilst GPs appreciated the intervention's self-management support principles, those with perceived high self-efficacy in gout management were less inclined to take up the intervention (Characteristics of individuals). Disseminating information on the intervention's efficacy to potential users of the intervention and engaging with intervention users through regular updates and feedback are strategies suggested for implementation (Process).
Discussion. We will use the findings to modify the intervention according to the GPs' needs and inform implementation strategies needed to ensure feasibility, appropriateness, and acceptability prior to piloting the intervention.

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Restriction of sodium in people with chronic kidney disease treated with empagliflozin (RESPECT-EMPA): protocol of a randomised trial

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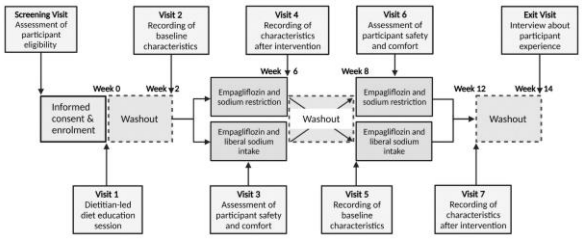
Introduction. Sodium-glucose cotransporter 2 (SGLT2) inhibitors and dietary sodium restriction are each known to have antihypertensive, renoprotective and cardioprotective effects in individuals with chronic kidney disease (CKD). However, the combined effect of the SGLT2 inhibitor, empagliflozin and dietary sodium restriction on 24-hour ambulatory blood pressure in people with CKD is not known.
Primary Aim. To examine the effect of empagliflozin and dietary sodium restriction, compared with empagliflozin alone during liberal sodium intake, on 24-hour ambulatory blood pressure in people with stage 1-4 CKD.
Methods. RESPECT-EMPA is an investigator-initiated prospective, single-centre, open-label, randomised crossover pilot study with a follow-up period of 14 weeks. A total of 30 participants will be recruited. Participants will be randomly assigned to either empagliflozin 10 mg daily with dietary sodium restriction (targeting 50 mmol of sodium per day) or empagliflozin 10 mg daily with liberal sodium intake for the first arm of the study. They will then cross over to the alternative sodium diet for the second arm, following a two-week washout period. Each treatment arm will last four weeks.
Discussion. It is hypothesised that in people with stage 1-4 CKD, the combination of empagliflozin and dietary sodium restriction will result in a greater reduction in mean 24-hour ambulatory systolic blood pressure than empagliflozin alone during liberal sodium intake. Results from RESPECT-EMPA will be used to inform larger randomised controlled trials.



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Exploring the binding kinetics of α_1-adrenoceptor ligands

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Introduction. The α_1-adrenoceptors (AR) have great therapeutic potential yet achieving subtype selectivity is still a challenge. However, ligand kinetic binding properties may identify variations across the subtypes and present an alternative for achieving subtype selectivity. This requires measurement of the temporal behaviour of ligands at α_1AR.
Aims. To determine binding kinetic parameters of adrenergic ligands at the α_{1A} and α_{1B} AR.
Methods. [^{3}H]-prazosin dissociation, and two-concentration and competitive association binding assays were conducted with $\alpha_{1A/1B}$AR membranes, and unlabelled noradrenaline (NA) to determine both drugs' association (K_{on}) and dissociation (K_{off}) rates. Data was analysed using three models: Motulsky & Mahan (1984); an extension of Motulsky & Mahan by Hoare (2021) that accounts for rapidly dissociating compounds; and Guo et al (2013)'s dual-point competition Kinetic Rate Index (KRI) method.
Results. Binding kinetic values for [^{3}H]-prazosin at $\alpha_{1A/1B}$AR ($\log K_{on}$ 1A 8.3±0.3; 1B 8.1±0.1, p=0.6; $\log K_{off}$: 1A -1.2±0.3; 1B -1.8±0.2, p=0.1, n=4-5) were not significantly different when calculated from association assay data. In contrast, [^{3}H]-prazosin dissociation assay data was 8-fold decreased at α_{1B}AR compared to α_{1A}AR. NA had increased K_{off} at α_{1A}AR compared to [^{3}H]-prazosin ($\log K_{off}$ -0.5±0.2 n=5 p=0.02). For α_{1B}AR, two of the four assays yielded unrealistic K_{on}/K_{off} values for NA. K_i's for both ligands were similar using Hoare and Motulsky & Mahan models. Meanwhile, calculated KRI values were dose-dependent, at odds with the characterisation of fast vs slow ligands per Motulsky & Mahan.
Discussion. Relative rates of association and dissociation of [^{3}H]-prazosin at the $\alpha_{1A/1B}$AR are quite similar, which is at odds with dissociation assay data. Furthermore, the models often failed to fit NA data obtained from α_{1B}AR but not the α_{1A}AR. We hypothesise that the Motulsky & Mahan (1984) method may struggle to fit compounds with lower affinities and produce unrealistic kinetic values, evident in the K_{off} of NA with α_{1B}AR.

Guo D et al (2013) J Biomol Screen 18:309-320
Hoare SRJ (2021) SLAS Discovery 26:835-850
Motulsky HJ and Mahan LC (1984) Mol Pharmacol 25:1-9

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Incomplete apoptosis in non-stimulated MDA-MB-468 breast cancer cells

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Introduction. Executioner caspase activation has been widely accepted as the 'point of no return' in apoptosis (Galluzzi et al, 2018). However, several reports reveal that cancer cells treated with apoptotic stimuli can escape cellular demise, even after executioner caspase activation. This process of incomplete apoptosis also endows surviving cells with more aggressive traits (Berthenet et al, 2020, Seervi et al, 2019). However, the propensity of cancer cells to undergo incomplete apoptosis has not been explored in non-stimulated (basal) conditions.
Aims. To assess the recovery of triple-negative MDA-MB-468 breast cancers from executioner caspase activation, under non-stimulated condition.
Methods. An MDA-MB-468 cell line with stable expression of the VC3AI genetically encoded caspase-3/7 indicator was generated (MDA-MB-468-VC3AI). MDA-MB-468-VC3AI cells with activated caspase-3/7 were harvested using fluorescence activated cell sorting (FACS). The sorted caspase-3/7 positive population was stained with a small molecule dye (CellTracker™ Red CMTPX) designed to label live cells. Cellular recovery from this population was assessed over time, using automated epifluorescence microscopy (ImageXpress).
Results. Caspase-3/7 positive cells (~ 0.5%) were detected in the MDA-MB-468-VC3AI cell population, in the absence of apoptotic stimuli. Caspase-3/7 positive cells successfully adhered to the plate one h post-FACS. Executioner caspase activation resulted in apoptotic cell death in most cells. However, a small number of caspase-3/7 positive cells successfully recovered and started proliferating. Colony formation and growth were observed at day 5, 7 and 10.
Discussion. This study suggests that executioner caspase activation is not an all-or-nothing checkpoint in apoptosis. Triple negative MDA-MB-468 breast cancer cells can undergo incomplete apoptosis, in the absence of apoptotic stimuli. Executioner caspase activity may be hijacked by cancer cells to drive cellular remodelling, rather than death.
Galluzi et al (2015) Cell Death Differ 22:58-73
Berthenet et al (2020) Cell Rep 31:1-14
Seervi et al (2019) Cell Oncol 42:645-61

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Relative contributions of GIPR activity in dual GLP-1R/GIPR agonist effects on whole-body metabolism

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Introduction. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones involved in the regulation of glucose and energy homeostasis. An emerging therapeutic approach involves combinatorial pharmacology to develop agonists targeting both GLP-1 receptor (GLP-1R) and GIP receptor (GIPR), which have shown profound weight loss, glycaemic control and lipid lowering effects. Despite efficacy of this approach, the role of GIPR activity in the superior metabolic benefits seen with these dual agonists over GLP-1R mono agonists remains unclear.
Aim. This study compares selective GLP-1R agonist Semaglutide with two dual GLP-1R/GIPR agonists; (NNC00902746 (Peptide 19) which has similar affinity for both receptors, and LY3298176/Tirzepatide (LY), which has higher affinity for the GIPR than the GLP-1R), with dosing designed to elicit similar occupancy of the GLP-1R with varying occupancies of the GIPR. We aim to identify the mechanisms of actions in establishing normoglycaemia and weight loss mediated by the GLP-1R/GIPR dual agonists, and if the weight loss effects are caloric intake-dependent through pair-feeding.
Methods. Male C57BL/6 mice were fed a high fat diet (HFD) or a control diet for 12 weeks, then weight matched before starting pair-feeding and chronic peptide treatment for 4 weeks. Ad libitum fed DIO mice were subcutaneously injected with either GLP-1R agonist (Semaglutide), or dual GLP-1R/GIPR agonists (Peptide 19 and LY) every 48 hours. Dosing for these studies was designed taking into account the pharmacokinetic profiles, and the affinity and potencies of each agonist at the GLP-1R. Metabolic assessments (e.g. blood glucose, insulin, body weight and composition, energy expenditure) were taken throughout the treatment period, with tissue collection at the end of the study.
Results. The treatment groups elicited varying levels of improvements in their metabolic profiles through differential degrees of GIPR activation. LY-treated mice demonstrated the greatest improvements in metabolic parameters and weight loss effect due to its highest degree of GIPR agonism. Food intake-independent effects of each treatment group were also observed when comparing the treated groups with their pair-fed groups.
Conclusion. GIPR activity plays a key role and contributes to the efficacy of the dual GLP-1R/GIPR agonists, improving metabolic homeostasis in a food intake-dependent and independent manner.

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Triple negative breast cancer: Screening for the invasion amplifying cAMP-calcium feedforward loop mechanism

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Introduction. Previously, we identified a cAMP-calcium (Ca^{2+}) feedforward loop mechanism in the highly metastatic triple negative breast cancer (TNBC) tumour cell line MDA-MB-231^{HM} (Pon et al, 2016). This mechanism facilitates the dynamic interplay between cAMP and Ca^{2+} second messenger systems following β_2 adrenoceptor activation, to further amplify both signals. Activation of this feedforward loop facilitates accelerated invasion in MDA-MB-231^{HM} cells.
Aims. To determine the commonality of the β_2-adrenoceptor mediated feedforward mechanism amongst a panel of TNBC tumour cell lines and to establish its role in regulating cellular invasion.
Methods. Formoterol was used to activate the endogenously expressed β_2-adrenoceptor in a panel of 10 TNBC cell lines. Relative mRNA expression of β-adrenoceptor subtypes was determined using qRT-PCR. Receptor signalling was measured using cAMP accumulation and Ca^{2+} mobilisation assays in the presence of a Ca^{2+} chelator (BAPTA-AM) or an adenylyl cyclase inhibitor (2',3'-dideoxyadenosine), respectively. Cellular invasion and proliferation were assessed using microscopy.
Results. Preliminary experiments identified both elevated cAMP and increased intracellular Ca^{2+} in response to β_2-adrenoceptor stimulation by the selective agonist formoterol in 5 out of 10 TNBC cells: MDA-MB-468 (pEC_{50} cAMP 9.22 ± 0.16, Ca^{2+} 8.82±0.23), HCC1143 (pEC_{50} cAMP 8.83±0.16, Ca^{2+} 10.13±0.09), HCC1806 (pEC_{50} cAMP 8.79±0.08, Ca^{2+} 8.70±0.89), HCC1395 (pEC_{50} cAMP 7.97±0.23, Ca^{2+} 8.76 ± 0.51), BT-549 (pEC_{50} cAMP 8.07±0.22, Ca^{2+} 10.73 ± 0.96). BAPTA-AM and 2',3'-dideoxyadenosine inhibited the cAMP and Ca^{2+} signals, respectively, suggesting that a cAMP/Ca^{2+} feedforward loop exists in these cells. Activation of the cAMP/Ca^{2+} feedforward loop correlated with accelerated invasion following β_2-adrenoceptor stimulation.
Discussion. The β_2-adrenoceptor can accelerate breast cancer progression in response to stress. The feedforward loop may provide strategies to more specifically target this GPCR in order to slow cellular invasion and metastasis.
Pon CK et al (2016) FASEB J 30:1144-1154

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GPR146: An orphan G protein-coupled receptor with atypical Class A motifs.

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Introduction. GPR146 is a Class A orphan G protein-coupled receptor (GPCR) linked to atherosclerosis and hypercholesterolaemia, but its pharmacology is unknown. We have observed that GPR146 features unique variation in some canonical Class A motifs that might affect receptor expression and/or activity. We hypothesise that variation in these critical residues may alter the behaviour of the receptor.
Aims. To use sequence alignments and literature searches to systematically analyse the potential functional implications of atypical residues within GPR146 motifs.
Methods. Human GPR146 sequence was aligned with 64 species orthologues (Ensembl) and 289 human non-olfactory Class A GPCRs (GPCRdb) using Discovery Studio Visualise 2019. Frequency of variation of canonical residues in motifs was compared for each set alignment.
Results. Highly conserved Class A GPCR residues vary in GPR146 from conserved motif sequences. The expected or most highly conserved residues in each motif are shown in the table alongside the GPR146 counterpart, with relative conservation in class A GPCRs indicated in parentheses. A2.50 is particularly unique, this being the only GPCR to feature a hydrophobic alanine at the 2.50 position, with A3.39 and S7.45 also varying from expected residues. The H3.50 of the DRY motif and the T7.49 of NPxxY also feature extremely rare substitutions but have comparatively similar residue properties. CWxP and PIF motifs, not shown, also feature some variation from canonical residues.
Discussion. The divergence of conserved residues of GPR146 compared to other Class A GPCRs may impact the function of the receptor. Because the affected motifs are known to be involved in signal propagation for many Class A GPCRs, future work should include mutagenesis studies to interrogate their impact on receptor activation.

Class A Motif (conservation %)	Human GPR146 (conservation %)
Na^+ binding site	
$D^{2.50}$ (92)	$A^{2.50}$ (0.35)
$S^{3.39}$ (71.3)	$A^{3.39}$ (3.1)
$N^{7.45}$ (66.1)	$S^{7.45}$ (11.1)
DRY	
$D/E^{3.49}$ (64.7/21.1)	$D^{3.49}$
$R^{3.50}$ (94.1)	$H^{3.50}$ (0.69)
$Y^{3.51}$ (66.4)	$Y^{3.51}$
NPxxY	
$N^{7.49}$ (72), $P^{7.50}$ (93.8), $Y^{7.53}$ (88.9)	$T^{7.49}$ (2.1) $P^{7.50}$ $Y^{7.53}$

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M4344, a potent and selective ATR inhibitor, synergises with radiation and temozolomide in patient-derived glioblastoma cells.

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Introduction. Patients with glioblastoma are confronted with a high likelihood of recurrence and poor prognosis despite an aggressive treatment-regime. Such standard treatment involves the use of radiation therapy (RT) and temozolomide (TMZ) to cause extensive DNA damage and replication stress, thus activating tumour cell death pathways. The upregulation of DNA repair mechanisms significantly reduces effective treatment response and contributes to poor patient outcomes.
Aims. To investigate the feasibility of inhibiting ATM- and Rad3-Related protein (ATR), a crucial sensor of replication stress and initiator of cell cycle arrest in tumour cells, using the potent and selective ATR inhibitor, M4344.
Methods. Twelve patient-derived glioblastoma cell lines were grown as monolayer cultures in serum-free media and treated with a clinically relevant dose of temozolomide (35µM) and/or radiation (2Gy) and/or serially diluted concentrations of M4344 (1000-31.25nM). The cell viability of treated glioblastoma cells was assessed after a 7-day incubation using the MTT assay.
Results. M4344, at concentrations from 1000nM to minimally cytotoxic concentrations of 125nM, significantly reduced cell viability when combined with either TMZ, RT or TMZ+RT compared to TMZ alone, RT alone or TMZ+RT, respectively, across all 12 cell lines. Notably, the ATR inhibitor synergised with TMZ and/or RT, increasing cell death of a recurrent patient-derived cell line (SB2b). Live-cell imaging of M4344 (1 µM) treated cells with TMZ (35uM) and RT (2Gy) showed a significant increase in cell death and apoptosis compared to TMZ and/or RT alone.
Discussion. These data suggest the potential for ATR inhibitor use as an effective chemo- and radiosensitiser in glioblastoma patients, warranting further investigation.

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Differential signalling of the PAC1n and PAC1n-hip receptor splice isoforms

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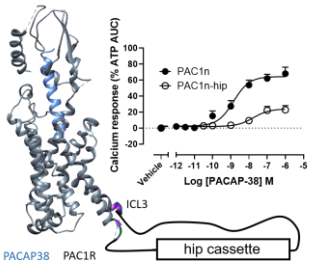

Introduction. The pituitary adenylate cyclase-activating polypeptide type I receptor PAC1R) is a potential therapeutic target for many CNS diseases (Vaudry et al, 2009). Multiple PAC1R splice isoforms exist, including PAC1n-hip which extends intracellular loop 3 (ICL3) by 28 residues (Lutz et al, 2006). ICL3 is critical for transducer coupling, hence ICL3 variants may alter transducer engagement and activation, altering downstream signalling. Here, we identified ligand-dependent differences in cAMP and Ca^{2+} signalling between PAC1n-hip and PAC1n (no ICL3 insertion), and in the coupling/activation and conformational profiles of two physiologically important transducers for PAC1R function; G proteins G_s and G_q.
Methods. COS-7 cells were transiently transfected with PAC1n or PAC1n-hip. cAMP and Ca^{2+} activity in response to agonists, VIP and PACAP38, were measured using a BRET-based cAMP sensor, CAMYEL, or a Ca^{2+} dye, fluo-8. $G_{s/q}$ activation was characterised using TRUPATH biosensors and ligand-mediated G protein conformation was assessed in membranes with BRET sensors; $G\alpha_{s/q}$-Nluc:$G\beta_1$:$G\gamma_2$-venus.
Results. Compared with PAC1n, PAC1n-hip had reduced E_{max} for PACAP38 and VIP mediated cAMP and Ca^{2+} signalling, but a selective decrease in PACAP38 potency (cAMP pEC_{50}: PAC1n-hip 8.1±0.1, PAC1n 9.3±0.1; Ca^{2+} pEC_{50}: PAC1n-hip 7.9±0.3, PAC1n 8.8±0.2, n=4–6, P<0.01). PAC1n-hip exhibited a decreased E_{max} without changing ligand potency in the TRUPATH G protein activation assay. G protein conformation analysis revealed differences in both the initial rate and degree of conformational change (PACAP38 G_q K_τ: PAC1n-hip 0.25±0.04 min^{-1}, PAC1n 0.44±0.04 min^{-1}, n=3, P<0.01).
Discussion. Insertion of the hip cassette into ICL3 resulted in reduced second messenger signalling, in addition to ligand-dependent signalling differences, which may be linked to slower G protein kinetics and distinct G protein conformations observed for PACAP38. This study provides new insights into PAC1R signalling.

Lutz EM et al (2006) Mol Cell Neurosci 31:193-209.
Vaudry D et al (2009) Pharmacol Rev 61:283-357.

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Shining a light on localised cAMP signalling and ERK phosphorylation using a targeted optogenetic GPCR.

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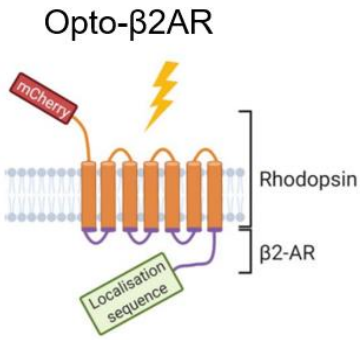

Introduction. The well-established paradigm of plasma membrane-restricted GPCR signalling is increasingly challenged by evidence of intracellular GPCRs that retain functionality. This spatial compartmentalisation of signalling results in location-specific production of second messengers that can confer unique downstream responses. This dynamic concept can be investigated using novel optogenetic methods which offer targeted and highly specific activation of intracellular signalling using light in lieu of ligands. Our approach utilised an optogenetic rhodopsin β2-adrenoceptor (opto-β2AR) chimera that couples to canonical Gs-mediated signalling in response to light (Siuda et al., 2015).
Aims. Target the opto-β2AR to various intracellular locations using novel location motifs and quantify light-mediated changes in cAMP, ERK phosphorylation and gene expression.
Methods. Subcellular GPCR localisation was validated using confocal microscopy; receptor activation was quantified using signalling assays and a quantitative reverse transcription polymerase chain reaction (qRT-PCR) array.
Results. Opto-β2AR was successfully targeted to early endosomes, Golgi, nucleus and mitochondria where light activation differentially increased cAMP accumulation and ERK phosphorylation. Activation of the opto-β2AR at each intracellular location also conferred unique responses at the transcriptional level.
Discussion. Disease-relevant GPCR signalling can be location dependant: a greater understanding of signal compartmentalisation will challenge existing conceptions about plasma-membrane delimited signalling and encourage new strategies for GPCR-targeted drug discovery.

Siuda ER et al (2015) Nat Commun 6:8480-8492

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RAMP modulation of calcitonin gene-related peptide (CGRP) family receptor function

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Introduction. Heterodimerisation of the calcitonin receptor-like receptor (CLR) and one of three receptor activity modifying proteins (RAMPs) yields three distinct receptors; the CGRP receptor (R), and adrenomedullin $(AM)_1R$ or AM_2R. These receptors are involved in vasodilation and protection from ischemia-reperfusion injury, while CGRP receptor antagonists are approved to treat migraine. The RAMP-dependent ligand selectivity of these receptors is best characterised at the canonical cAMP production pathway. Whether this is maintained across other signalling pathways is not well studied. Moreover, the contribution of distinct RAMP subdomains to the observed receptor phenotypes is largely unexplored.
Aims. To further understand the structural mechanisms underpinning RAMP modulation of receptor signalling.
Methods. Cos-7 cells were transiently transfected with CLR, wild type or chimeric RAMPs and various bio-sensors. Ligand-mediated Gs coupling, arrestin recruitment and receptor internalisation were monitored in real-time using bioluminescence resonance energy transfer (BRET) approaches.
Results. While CGRP can stimulate cAMP production at AMRs with lower potency than the cognate peptides, it is unable to trigger β-arrestin recruitment or receptor internalisation. Interestingly, in direct G protein-coupling assays, swapping the linker region of RAMP1 for RAMP2 reduces CGRP potency, but is without effect on AM peptides. But, swapping the RAMP2 linker for RAMP1 or RAMP3 distinctly increases potency for AM_2R mediated Gs-coupling. Intriguingly, Gs coupling was abolished for all ligands upon exchange of the short RAMP2 C-terminus with RAMP1.
Discussion. Our study revealed that the prototypical phenotype ascribed to the CGRPR, AM_1R and AM_2R is not retained across all functional and regulatory processes. In addition to inducing minimal AM_1R and AM_2R arrestin recruitment, CGRP also failed to replicate the “dissociation phase” of Gs coupling seen with AM peptides, suggesting that there may be a transducer protein recruitment threshold for initiating subsequent regulatory processes. The global importance of the RAMP2 C-terminus for AM_1R/Gs coupling is consistent with a potential role for this domain in the dynamics of G protein engagement.

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Activation of CC chemokine receptors CCR1 and CCR2 in THP-1 monocytes leads to reduced chemokine receptor expression and functional responses

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Introduction. The recruitment of monocytes to damaged or infected tissues is dependent on the activation of a subclass of G-protein coupled receptors known as chemokine receptors. Chemokine receptors are stimulated by the binding of chemoattractant cytokines (chemokines). In particular, inflammatory or classical monocytes are recruited by the binding of chemokines CCL2 and CCL5 to chemokine receptors CCR2 and CCR1 respectively. After reaching the target tissue, classical monocytes can differentiate into macrophages or dendritic cells.
Aims. To determine the changes in expression and function of chemokine receptors, and the expression changes of key phenotypic markers, in monocyte-like THP-1 cells after stimulation of CCR1 and CCR2.
Methods. Next-generation RNA-sequencing (RNA-seq) was performed on THP-1 cells that had been treated with CCL5 (ligand of CCR1), CCL2 (CCR2) and CCL7 (CCR1 and CCR2) separately for 24 hours. Changes in expression of chemokine receptor and marker transcripts were validated using qRT-PCR. Functional changes in the THP-1 monocytes, before and after chemokine treatment (24 hours), were detected using cell-based signalling (phosphoERK1/2; AlphaLISA Surefire Ultra, Perkin Elmer) and chemotaxis (96- well MultiScreen plates, Merck) assays.
Results. RNA-seq data showed that treatment of THP-1 monocytes with CCL2 for 24 hours resulted in decreased expression of both CCR1 and CCR2 transcripts. qRT-PCR data validated this result and further showed that 24-hour treatment with CCL5 or CCL7 also decreased the levels of CCR1 and CCR2 mRNA. In line with these expression changes, we also observed decreases in CCR1- and CCR2-mediated ERK phosphorylation and chemotaxis after a 24-hour treatment with CCL2.
Discussion. These data indicate that the migratory phenotype of THP-1 monocytic cells may be altered after activation of CCR1 or CCR2. Ongoing analyses of phenotypic markers will determine whether these cells are also beginning to differentiate. The biological significance of these data will be explored by conducting similar experiments in human primary monocytes.

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Enhanced nitric oxide production by macrophages treated with SPSB-iNOS inhibitors conjugated to cell-penetrating peptides

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Introduction. Nitric oxide (NO) is a key effector molecule of the innate immune response, and plays a crucial role in macrophage killing of infective agents.[1] NO is produced by inducible NO synthase (iNOS) in macrophages in response to foreign stimuli. The lifetime of iNOS is regulated by proteasomal degradation, which is mediated by binding to SPRY domain containing SOCS box proteins (SPSB).[2] Disruption of the iNOS-SPSB interaction resulted in enhanced lifetime of iNOS, increased NO production and pathogen killing.[2] A series of linear and cyclic peptide inhibitors of the iNOS-SPSB interaction have been designed based on the DINNN motif and refined for increased binding affinity, stability and drug likeness.[3-5]
Aims. Assess cellular uptake of cell penetrating peptide (CPP) conjugated SPSB-iNOS inhibitors and subsequently enhanced NO production by macrophages.
Methods. We have conjugated SPSB-iNOS inhibitors with CPPs and fluorophores. We performed confocal imaging to confirm cellular uptake. NO levels were measured using the Griess assay.
Results. We have demonstrated that the binding of CPP-conjugated inhibitors to SPSB is not compromised by this conjugation. We have confirmed the successful uptake of fluorophore-tagged inhibitor-CPP conjugates by RAW 264.7 and immortalised bone marrow derived macrophage (iBMDM) cell lines. We have designed and optimised an assay to evaluate the potential of CPP-cargo to enhance NO production and found that these inhibitors elevated NO production. We have also shown that these inhibitors are not toxic to macrophages.
Discussion. The findings of this study will be useful in further optimising the design of SPSB inhibitor-CPP conjugates.

1. Bogdan, C et al. (2000) Immunol. Rev. 173 :17-26. 2 Kuang, Z. et al. (2010) J. Cell Biol. 190: 129-141. 3. Yap, B. K. et al. (2016) FEBS Lett. 590: 696-704. 4. Yap, B. K. et al. (2014) J. Med. Chem. 57:7006-7015 5. Sadek, M. M. et al. (2018) ACS Chem. Biol. 13: 2930-2938

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Investigating the activity and role of store operated calcium entry (SOCE) in a cellular model of senescence

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Introduction. Calcium (Ca^{2+}) homeostasis dysregulation has been linked to many age-related diseases such as Alzheimer's, Parkinsons disease, Huntington's disease and many more. SOCE is one of the major pathways involved in the regulation of Ca^{2+} homeostasis intracellularly. Human derived fibroblasts (HDFs) have limited replicative capacity known as the Hayflick limit after which cells undergo growth arrest known as replicative senescence.
Aims. To study the activity of SOCE in cellular ageing and the effect of pharmacological modulation of SOCE on cellular senescence.
Methods. The activity of SOCE in young and aged HDFs was assessed in calcium influx assays using Fluo-4 calcium indicator. To understand the effect of SOCE modulation on cellular senescence, HDFs were treated with DMSO control or SOCE agonist, IA65 (1, 3 and 10 µM), or SOCE inhibitor, Synta-66 (1 and 10 µM) and cells were passaged at fixed time interval of four and three days. Cumulative population doublings (CPD) were calculated at each passage until the cells reached the plateau phase of cell growth (Passage 38).
Results. Calcium influx assays demonstrated that SOCE pathway is active in the HDFs and can be regulated by its pharmacological modulators, IA65 and Synta-66. In addition, HDFs treated with IA65 at 3 µM concentration showed a delayed plateauing of the CPD curve compared to the other treatment groups.
Discussion. SOCE is present in HDF and can be modulated using IA65 and Synta-66. Preliminary data shows that there is a modest effect of SOCE activator, IA65 at 3 µM, on delaying the onset of replication arrest, however further investigation is needed to confirm the effect of the drug on delaying cellular senescence. Given the implication of cellular senescence in various pathological conditions, identification of novel therapeutic targets against cellular senescence, may pave the way to treatment of these conditions.

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β-adrenoceptor Signalling in Pancreatic Cancer Cells

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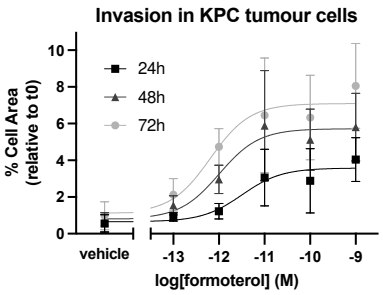

Introduction. The sympathetic nervous system drives pancreatic cancer progression through β-adrenoceptor receptor signalling, consequently making these receptors an emerging target to slow cancer progression. However, little is known about the specific subtype of β-adrenoceptor and the signalling pathways that are responsible for pancreatic cancer progression, or whether this occurs in all pancreatic cancer.
Aims. To determine the signalling pathway activated by different β-adrenoceptor subtypes in pancreatic cancer tumor cells, and the effect this has on cellular proliferation and invasion.
Methods. A mouse pancreatic ductal adenocarcinoma cell line (KPC) as well as two different human pancreatic ductal adenocarcinoma cell lines of varying differentiations (HPAF-II and Panc-1) were used. β-adrenoceptor expression levels were determined using qRT-PCR, signalling pathways were assessed using cAMP accumulation and calcium mobilisation assays, and cell proliferation and invasion were measured by imaging.
Results. All cell lines show an increase in cAMP and calcium in response to isoprenaline. The isoprenaline response is mediated by the β2-adrenoceptor in KPC and HPAF-II tumour cells, whereas both the β1- and β2-adrenoceptor contribute to the isoprenaline response in Panc-1 tumour cells. Activation of the β2-adrenoceptor accelerates invasion of KPC and Panc-1 tumour cells after 48 hours ($p=0.013$ and $p=0.012$, maximal response of KPC and Panc-1 tumour cells at 48h, respectively, vs vehicle control, one-way ANOVA with Šídák's multiple comparisons post-test, n=4-6), with no effect on cellular proliferation. In contrast, there was no effect of β-adrenoceptor stimulation on HPAF-II invasion. Instead, we observed an increase in cell proliferation after 24 hours ($p=0.002$, maximal response at 24h vs vehicle control, one-way ANOVA with Šídák's multiple comparisons post-test, n=5).
Discussion. The effect of β2-adrenoceptor activation on proliferation or invasion varies with cell type. A more detailed understanding of the differences in the signalling pathways that lead to either invasion or proliferation is of future interest.

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Examination of unbound plasma, total plasma and whole blood tacrolimus in elderly kidney transplant recipients

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Background: Therapeutic drug monitoring is routinely performed to maintain tacrolimus whole blood concentrations in a desired target range following kidney transplantation. However, variability in unbound plasma concentrations can result in patients experiencing toxicity with optimal drug levels. Aim: To compare paired unbound plasma concentrations (C_u), total plasma concentrations (C_p) and whole blood concentrations (C_{wb}) of tacrolimus in elderly kidney transplant recipients 4-6 weeks post-transplant.
Methods: Twelve-hour concentration-time profiling was performed in 15 elderly patients (>65 year) to measure tacrolimus C_u, C_p and C_{wb} values. Drug concentrations were measured by HPLC-MS. Based on a saturable binding model, C_{wb} was related to C_p and haematocrit (expressed as a fraction; fHCT) as follows:
$C_{wb} = C_p*(1+Bmax*fHCt / Kd+C_p)$; where Kd is the equilibrium dissociation constant and Bmax is the maximum binding concentration expressed per volume of erythrocytes. Based on linear binding kinetics, C_p was related to C_u as follows: $C_p = C_u/Nplasma$; where Nplasma is the non-specific binding constant . A non-linear regression analysis was performed to obtain best estimates of Bmax, Kd and Nplasma to describe the relationships between C_{wb}, C_p and C_u.
Results: 195 paired tacrolimus C_{wb}, C_p and C_u values were collected in total. The majority of tacrolimus was associated with erythrocytes, as C_p was <10% of C_{wb}, and the unbound fraction was <0.005. The median (range) of Bmax was 90.4µg/L (22.4 – 752.5), Kd was 2.4µg/L (0 - 69.2), and Nplasma 0.05 (0.035 - 0.085). Inter-individual variability (CV%) in the binding constants was considerable (Bmax 117.2%; Nplasma 32.5%).
Conclusion: Large variability was observed in tacrolimus binding constants and free plasma concentrations in elderly kidney transplant recipients. Future research examining the relationship between tacrolimus C_u and patient outcomes may be of benefit.

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Chemotherapy insensitivity in a Novel Plasma-like Culture Medium, the *Melbourne Medium*

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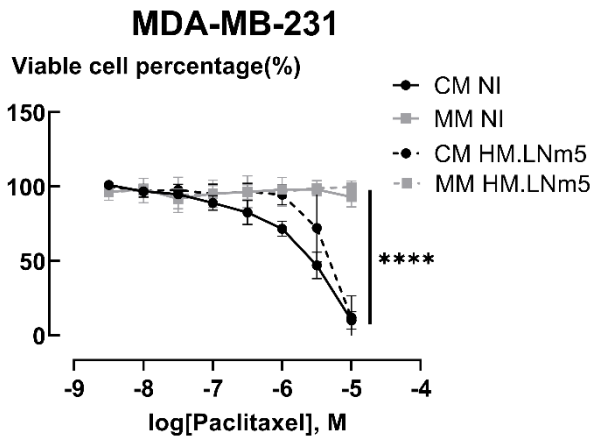
Introduction. The tumour microenvironment (TME) is a complex mixture of soluble and insoluble molecules influencing cancer progression. *Melbourne medium* (MM) is designed as a novel plasma-mimic medium for modeling the tumour soluble microenvironment. Conventional medium (CM), including DMEM, is a very commonly used cell culture medium developed from work in the middle of the last century to support cancer cell growth. CM is hyper-nutritious by comparison with plasma/interstitial fluid and may distort chemotherapy responsiveness.
Aims. To ascertain the influence of different culture media on chemotherapy responses of MDA-MB-231 tumour cells.
Methods. Operetta high-content-imaging was used to evaluate paclitaxel potency in the MDA-MB-231 (NI) cell line and the highly metastatic daughter line (HM. LNm5), measuring viability, proliferation and spontaneous migration.



Results. MDA-MB-231 cell lines in CM were sensitive to paclitaxel (3nM-10µM), whereas cell in MM appeared insensitive (n=5, mean and SEM, ****P<0.0001 repeated measures two-way ANOVA). This difference in chemo-sensitivity was also observed in human lung adenocarcinoma epithelial cell line A549 and human pancreatic adenocarcinoma cell line AsPC-1. Lower proliferation rates and higher spontaneous motility were observed in MDA-MB-231 (NI) cells in MM.
Discussion. Chemotherapy potency is strongly influenced by the culture media composition, as are cell proliferation and motility. These findings may shed light on the frequent translational discordance between outcomes of preclinical and clinical cancer treatment.

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Effects of chronic polypharmacy and monotherapy on metabolic ratio, clearance, and its association with functional outcomes in aged mice

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Introduction. Age-related physiological changes lead to changes in pharmacokinetic processes, such as drug metabolism and clearance. Polypharmacy (use of ≥5 medications) is common in older people, and may cause drug interactions and therefore alter pharmacokinetics. The effect of chronic polypharmacy on drug metabolic ratio and clearance is poorly understood. Pharmacokinetic calculations in a preclinical model can be used to understand these changes.
Aims. Using an aged mouse model, we investigated the effect of chronic polypharmacy and monotherapy on (i)metabolic ratio, (ii)clearance and (iii)its association with functional outcomes.
Methods. At 12 months old, male C57BL/6 mice received a chronic diet of either control feed, polypharmacy (metoprolol, simvastatin, citalopram, oxycodone, oxybutynin) at therapeutic doses, or one of the five drugs as monotherapy (n=30-40/group). Serum drug concentrations, dose and functional outcomes were measured at 15, 18, 21 and 24 months of age. Pharmacokinetic calculations were performed to calculate metabolic ratio and total clearance (CL/F), which were correlated with relevant functional outcomes.
Results. Polypharmacy treatment increased the parent drug to metabolite ratio compared to monotherapy with metoprolol for every age group (15m polypharmacy mean=10.70, monotherapy mean=2.40, $p<0.05$). Drug clearance was reduced in the polypharmacy treatment group compared to monotherapy with metoprolol and citalopram (18 months: metoprolol: polypharmacy mean=0.02g/L/h, monotherapy mean=0.10g/L/h, $p<0.05$; citalopram: polypharmacy mean=0.59g/L/h, monotherapy mean=1.82g/L/h, $p<0.05$). Metoprolol clearance did not correlate with blood pressure or rotarod endurance time ($p>0.05$).
Discussion. Our results suggest that polypharmacy may impair drug total clearance, beyond what is anticipated by knowledge of drug pair interactions. Future research will characterise specific hepatic enzyme activity and protein expression changes caused by polypharmacy.

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Exploring healthcare students motivations during online learning in the COVID-19 pandemic

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Introduction. Restrictions imposed by the COVID-19 pandemic severely disrupted education across the world in 2020 and 2021. Students have reported various difficulties in learning during COVID-19 restrictions. This is especially of concern for healthcare students, who often had their critical in-person practical classes disrupted by the restrictions. There is little research currently that examines how the changes in learning for healthcare students has affected their motivation to study during COVID-19.
Aims. To investigate the factors affecting motivations for learning in healthcare students undertaking emergency remote learning during COVID-19, using the Self-Determination Theory framework.
Methods. A multi-database, scoping systematic review was undertaken to examine literature published from Jan 2020 to June 2021 examining articles that described changes in motivation in healthcare students globally based on changes to the types of methods of delivery for learning. Further, a survey was distributed to healthcare students at The University of Sydney (HREC No. 2011/611) who were enrolled in both years 2020 and 2021. The survey compared the type of methods of delivery that students were exposed to, to the type and severity of disruptions they experienced and their satisfaction of the psychological needs of competence, autonomy and relatedness.
Results. Phase I of the systematic scoping review identified 126 articles of interest in a search of 1096 articles, from a range of developed and developing nations. Phase I identified multiple different synchronous and asynchronous remote learning techniques for both theory and practical content, with varied levels of motivational satisfaction. Phase II of the review, as well as the survey and data collection are still underway at the time of preparing this abstract.
Discussion. COVID-19 restrictions have had a complex effect on the learning of healthcare students. We anticipate our findings will show a preference for a mix of different remote learning techniques that promote competency and relatedness whilst maintaining autonomous learning so that educators can better manage student motivation during potential future restrictions requiring remote learning.

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Lecture recording use and association with academic outcomes in a pharmacology course

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Introduction. Surveys of lecture recording use by students in pharmacology courses are available, but not direct data studies.
Aims. For students studying pharmacology in a biomedical science program in 2019, to quantify direct data of how lecture recordings were accessed, and any association between accessing lecture recordings and academic outcomes.
Methods. The number and duration of accesses to lecture recordings were determined weekly, and cumulatively.
Results. Lectures were at 8 am, and attendance was very low. Workshop attendance declined during semester. Fifty-one of 66 students consented to the study (77%), and 47 of these accessed lecture recordings via Blackboard. Only 14 students accessed all 9 lectures. The number of lectures accessed averaged 6.3. Most of the students did not access the recordings in their entirety in one access, and the number of accesses average 2.4/lecture recording. The numbers of students accessing lecture recording in the week after they were posted decreased throughout the semester. For the lectures in the first three weeks of semester, most students accessed the lectures shortly after the lecture was posted. However, for later lectures, most students did not access the recordings accessed until the three weeks prior to the examination. Regression analysis of academic outcome (grade, overall mark, ongoing assessment, and exam) versus average lecture recording accessed (minutes) showed no association. However, there was a significant positive association between the grade, overall mark, and exam mark for lecture recording access in the three weeks after presentation (r =3-4). In contrast, there was no association between academic outcomes for the access in the three weeks before the examination.
Discussion. As the ongoing assessment in this course (research plan and literature review) was not linked to lecture content, it is not surprising that the marks in these were not associated with lecture recording access. The most interesting finding of this study was that the lecture recording access soon after the lecture was posted was positively associated with exam marks, whereas lecture recording access prior to the exam was not. A possible explanation for this is that early engagement with lecture recording is more effective than late (cramming) access to the recordings.

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Impact of delivery modes during COVID-19 on student attainment of learning outcomes in pharmacology

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Introduction. COVID-19 pandemic has significantly disrupted the ways in which students and educators engage in learning, necessitating transformation of once entirely face-to-face curricula to wholly online or hybrid-flexible (HyFlex) modes, where classes are run concurrently online and on campus. This has raised concern around education of some of the valued practices of disciplinary work and attainment of learning outcomes relevant to pharmacology.
Aims. To investigate the potential impacts of remote learning on student performance in second year pharmacology fundamentals units of study.
Methods. Students enrolled into second year pharmacology fundamentals units of study learnt either remotely or on campus in semester 1, 2021. Marks and grades for each cohort of students were analysed for similarities or differences in overall performance on the basis of enrolment mode and international student status using two-way ANOVA followed by post-hoc contrast analyses. (HREC approval number 2020/450).
Results. Students who attended classes on campus performed significantly better than students who were enrolled remotely. Moreover, international students learning remotely were at a particular disadvantage.
Discussion. Multiple factors influence student learning and performance, however it is evident that remote learning poses barriers to student attainment of learning outcomes, at least in the way delivery of valued disciplinary practices was designed for remote learners here. Further research is needed to identify barriers and solutions in remote delivery methods to inform future educational design for more inclusive and engaging learning of valued disciplinary practices.

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Partnering with students: reflections from an academic-led support program for international students

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Introduction. Partnering with students refers to meaningful cooperative, collaborative and mentoring relationships between students and staff members at a university (1). Faculty-based student leadership programs such as the Ambassador Program at the Faculty of Pharmacy and Pharmaceutical Sciences (FPPS) at Monash University, offers students a host of opportunities to develop leadership, teamwork, public speaking, and mentoring skills, and present an opportunity for cultivating innovative partnerships between staff and students.
Aims. To partner with the Ambassador Program at FPPS to co-design, develop and implement extra-curricular events as part of an academic-led support program for international students.
Methods. Six international students as part of the Ambassador program were selected and partnered with academics at FPPS leading an academic support program for international students. Under the guidance and mentorship of the academic team, these students designed, developed, and implemented three activities to engage the undergraduate international cohort at FPPS. The partnership was sustained over the academic year.
Results. The student perspective was insightful in designing 3 well-being, networking and support events to engage the international student cohort. Partnering with the student ambassadors increased the reach of the academic-led support program and facilitated event advertisement on student platforms such as student committee and year level Facebook and Instagram accounts. The students were resourceful in finding unique ways to make the international students feel connected, valued and part of the Monash community - for example sourcing prizes such as Monash hoodies including seeking approval from Faculty to cover postage and handling to send hoodies to those offshore.
Discussion. This unique partnership was mutually beneficial for both staff and students. Students received mentorship from the academic team to develop their leadership skills and staff were able to deliver events through the lens of the students which further increased interest and engagement within the international student cohort.
1. Matthews et al. (2018). Connecting learning, teaching and research through student-staff partnerships. In Tong et al (Eds.), Shaping higher education with students: Ways to connect research and training. London: UCL Press

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Can multiple choice questions assess higher cognitive thinking? A comparative study of students' performance in online vs invigilated exams

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Introduction. Application of knowledge is one of the core attributes of graduates from pharmacy schools and related disciplines in Australia and worldwide.
Aims. The aim of this study is to examine the use of multiple choice questions (MCQs) to assess the application of knowledge and to compare if students' performance in different cognitive level MCQs is consistent in online vs invigilated exams.
Methods. MCQs were developed and ranked into one of two cognitive levels, based on a modified Bloom's taxonomy, as knowledge recall 'KQ' or application of knowledge 'AQ'. Ranked MCQs were included in the mid-semester exam and the final exam of a Pharmacology course. Student performance on MCQs was compared between and within each Bloom's level, as well as between online vs invigilated MCQs over 3 years. The differences in the percentage of students who obtained a correct answer for each level were then analysed using Student's *t* Test.
Results and Discussion: An average of 60 students were enrolled in the Pharmacology course each year for 2018 to 2020. Ninety five MCQs (comprised of ~37 KQ vs 58 AQ) were included in the final exam and forty MCQs (comprised of ~12 KQ vs 28 AQ) were included in the mid-semester exam over the 3 years. Mean average score for online AQ was 87.0% compared to 97.5% for KQ in the final exam ($p = 0.5$). Similar results were found for the mid-semester exam, with an average score of 85.9% for online AQ compared to 92.1% for KQ ($p = 0.28$). Mean average score for invigilated AQ was 66.3% and 65.1% compared to 76.0% and 77.3% for KQ in the 2019 and 2018 final exam ($p = 0.30$; 0.35), respectively. Overall score for online MCQ in the mid semester exam was 87.8% compared to an average grade of 76.9% and 76.8% for invigilated exam in 2019 and 2018 ($p = 0.31$; 0.39) respectively. Overall, student performance for AQ was consistently reduced by roughly 10% score compared to KQ in all exams. In addition, it was interesting to see that online MCQs were scored roughly 20% higher across all Bloom's level MCQs as compared to that in invigilated exams.
Conclusion: In summary, well-designed MCQs which target various cognitive levels can be used in pharmacology exams, online and invigilated, to facilitate assessment of student performance and higher cognitive thinking.

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Hybrid teaching and learning: Technology and tools to facilitate students engagement

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Introduction. The COVID-19 has created unique challenges to the education sector across the globe in early 2020 as well as the later years. Although face-to-face classes can be resumed in semester 1 2021 as restrictions lifted, hybrid and blended delivery modes begin to emerge and become an important integration at university teaching and learning.
Aims. The aim of this study was to describe issues of transition to face-to-face delivery of a pharmacology course and evaluate the technology and tools necessary for successful hybrid delivery.
Methods. To address compulsory face-to-face attendance for 2 of 3 course class activities, including practical and tutorial per university policy but proven impractical due to diversity in students demographics plus timetable anomalies, course co-ordinator defined the scope of face-to-face delivery issue and proposed feasible delivery mode for the affected activity.
Results and Discussion. As course coordinator is not given flexibility to move classes on-line spontaneously, the affected class (tutorial) was remained face-to-face, however live-streamed and recorded to accommodate those unable to attend face-to-face. Use of whiteboard became problematic as available technology at institution does not capture whiteboard's activity. Thus, alternative tools were explored to replace the use of whiteboard. Briefly, Echo 360 was used for live streaming and recording of face-to-face tutorial, with recording published on-line once class finished. Students were given the flexibility either to attend tutorial face-to-face or live-stream in. Compulsory attendance was waived by course coordinator, after consultation with HOS/program director. A document projector was used to replace whiteboard. All teaching material for tutorial was provided on-line prior to tutorial.
Conclusion. Course with flexible delivery mode appears to be of high demand at present, also attracts student enrolment. Suitable technology is extremely important to facilitate student engagement in hybrid teaching. Contents can be easily delivered on-line via live-streamed and recorded face-to-face class, however it is difficult to engage successfully both audiences. More advanced technology and tools are necessary for successful hybrid delivery e.g. use of hybrid teaching room. Hybrid delivery generally requires more time and preparation, educators often face more challenges, these challenges include increased workload in all aspects of course coordination and delivery.

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Promoting more efficient in-semester revision in medical pharmacology using spaced repetition

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Introduction: Improving medical student competency in pharmacology is crucial as the discipline underlies safe and rational prescribing practice. A learning technique of interest is spaced repetition, which involves personalised scheduling of flashcard revision based on the user's previous difficulty ratings. Use of spaced repetition has been shown to improve student outcomes, although literature regarding use in pharmacology education is lacking.
Aims: This project aimed to design and implement a spaced-repetition revision program for medical students and evaluate student perceptions and engagement with the resource
Methods: Utilising student input from previous surveys and focus groups, a set of 1208 flashcards spanning 156 distinct classes of drugs with supplementary summary tables and diagrams were designed to provide students with time-efficient, concise, and relevant learning resources for revision. This resource was released to approximately 100 medical students, with evaluation was performed utilising a mixed methods approach utilising a survey incorporating Likert scale and free-text items.
Results: Evaluation by 29 participants showed that students appreciated the "comprehensive," and "well formatted," resource which supported existing teaching. Students rated the spaced repetition resource 3.7 out of 5, with 76% planning to utilise the resource long term and 83% requesting the development of similar resources for other subjects.
Discussion: This study has illustrated that spaced repetition resources are well accepted and successfully engage students in consistent revision that underlies academic success. Ultimately, this may improve medical student knowledge and application of pharmacology.

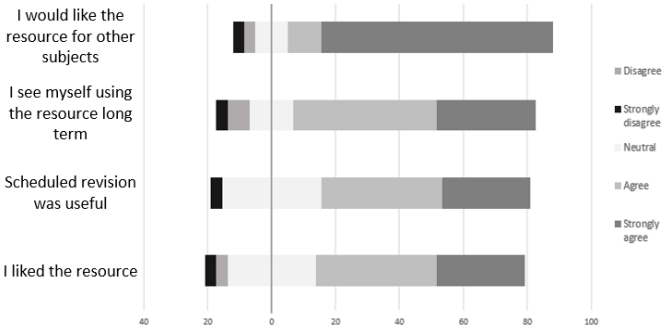

Figure 1: Likert scale evaluation of the spaced-repetition resource

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Effects of co-treatment with common medications on paracetamol and olanzapine entry into developing rat brain

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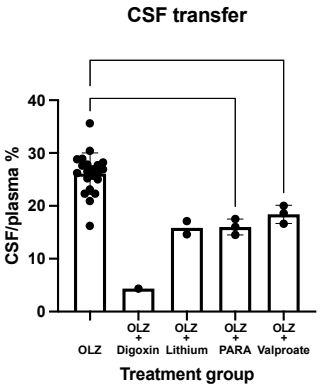

Introduction. Paracetamol is one of the most commonly used drugs with over 70% of women taking it even during pregnancy (Werler et al., 2005), while many women with psychiatric disorders at childbearing age need to continue medicating throughout pregnancy and lactation as cessation could be dangerous for both mother and child. Information on transfer of these drugs into the developing brain is limited.
Aims. i) To measure transfer of paracetamol (PARA) and olanzapine (OLZ) across placental and blood-brain barriers in rats during development following acute mono-treatment. ii) To determine age-related effects of co-treatment with common medications (paracetamol, olanzapine, cimetidine, digoxin, fluvoxamine, lithium, lamotrigine or valproate) at clinical doses on paracetamol and olanzapine permeability.
Methods. Sprague Dawley rats were injected i.p. with 15mg/kg paracetamol or 0.15mg/kg olanzapine with respective radioactive tracer at 3 ages (E19, P4, adult), either alone or in combination with other drugs. Transfer of paracetamol and olanzapine into brain and CSF after 30min was measured using liquid scintillation counting and LCMS.
Results. An age dependent decrease in transfer into both brain and CSF (~65% at E19 and P4 to ~30% in adult) was observed after paracetamol exposure. Olanzapine transfer remained stable in brain (~75-100%) throughout development, however decreased in the CSF (from ~25% at E19 and P4 to 12% at adult). In pregnancy, around 40% of the drug was transferred from the maternal blood to the fetal circulation for both drugs. Co-administration with olanzapine increased paracetamol permeability into brain and CSF, whereas olanzapine permeability decreased in the developing brain and CSF following combination with digoxin, lithium, paracetamol and valproate (see figure above).
Discussion. Olanzapine and paracetamol were able to enter the fetal brain in spite of placental protection. In addition, co-administration of some commonly used drugs appeared to affect their entry into the developing brain.

Werler et al (2005) Am J Obstet Gynecol 193:771-777

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Can the stroke recovery effect of zolpidem be mediated via alternative benzodiazepine binding sites?

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Introduction. Movement disorders and language impairment are common complications observed in stroke survivors. These complications are often debilitating and require extensive therapy. To date, a few reports have demonstrated that zolpidem, a sedative $GABA_A$ receptor ($GABA_AR$) modulator, paradoxically improved cognitive, language and other stroke-induced impairments (Sutton et al, 2017). As the sedative effect of zolpidem is believed to be mediated through benzodiazepine-binding site in the α1(+)γ2(-) interface (Crestani et al, 2000), we hypothesised that the effect of zolpidem in improving post-stroke complications may be mediated via alternative benzodiazepine binding sites.
Aims. To determine whether zolpidem can modulate $GABA_AR$ via α1(+)α1(-), α1(+)β2(-) and α1(+)δ(-) interfaces that are similar to the α1(+)γ2(-) interface and potentially contain alternative benzodiazepine-binding pockets.
Methods. Site-directed mutagenesis was conducted to change key residues on the γ2 subunit such that the mutated subunits mimic the subunit (-)-face of α1, β2 and δ subunits. Mutant γ2 subunits were expressed with wild-type α1 and β2 subunits on *Xenopus* oocytes and two-electrode voltage clamp experiments were used to determine GABA and zolpidem concentration-response relationships.
Results. Receptors with $\gamma2^{\alpha1(-)}$, $\gamma2^{\beta2(-)}$ and $\gamma2^{\delta(-)}$ mimics all produced significant reduction in zolpidem potency, with zolpidem EC_{50} values of 3.1μM, 21μM and 130μM, respectively, compared to the wild-type γ2 subunit (0.23μM).
Discussion. Our study showed that zolpidem potency is most clinically relevant at the classical α1(+)γ2(-) benzodiazepine interface, and the binding of zolpidem via alternative α1(+)α1(-), α1(+)β2(-) and α1(+)δ(-) interfaces are unlikely to elicit significant allosteric modulation of $GABA_AR$ at clinically relevant concentrations. Hence, we demonstrate that the zolpidem's effect in alleviating stroke-induced complications is unlikely to be mediated via alternative $GABA_AR$ benzodiazepine sites.

AIHW (2020) Stroke, Canberra, Australian Institute of Health Welfare
Sutton JA, Clauss RP (2017) Brain inj 31(8):1019-1027
Crestani F et al (2000) Br J Pharmacol 131(7):1251-1254

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Investigating Functional Bias at the Tachykinin Receptor Family

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Introduction: Biological processes of tachykinin peptides are mediated by a family of three neurokinin G protein- coupled receptors. Previous studies suggest that the neurokinin-1 and neurokinin-2 receptors (NK1R/NK2R) can signal through multiple signalling pathways, such as the Gq and Gs pathways leading to the mobilisation of intracellular calcium and formation of cAMP, respectively.
Aims: We aim to elucidate the signalling landscape of NK1R and NK2R upon activation from endogenous tachykinins and investigate potential functional selectivity.
Methods: The peptides substance P (SP), neurokinin-A (NKA), neurokinin-B (NKB), and hemokinin-1 (HK-1) were tested in multiple assays including intracellular calcium mobilisation, inositol monophosphate accumulation (IP1), and cyclic AMP production using Flp-In-HEK293 cells stabling expressing the human NK1R and NK2R. Additionally, recently developed BRET2-based biosensors were employed to illuminate G protein activation at the tachykinin receptor family.
Results: At the NK1 receptor SP, NKA, NKB, and HK-1 were potent agonists in stimulating IP1 accumulation and activating Gq. We found that NKA and HK-1 were just as potent as SP in cyclic AMP accumulation assays and in intracellular Ca^{2+} mobilisation. Analysis using the operational model of agonism revealed that NKB was biased away from the Ca^{2+} and cyclic AMP pathways with reference to SP. It was demonstrated at the NK2 receptor that NKA was the most potent agonist in comparison to the other endogenous ligands. This falls in agreement with the rank order of preferential binding at the NK2 receptor (NKA > NKB > SP). Using the BRET-2 biosensors, both receptors were found to activate Gq yet activation of Gs was not detected.
Discussion: At the NK1 receptor, NKA and HK-1 are just as potent agonists as SP (the preferred ligand for NK1), whilst NKB displays functional selectivity. These results provide insights into the signalling landscape of the tachykinin receptors, which will aid understanding the pharmacological properties of endogenous ligands and potential therapeutic outcomes.

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Effect of chronic polypharmacy on motor balance and coordination in old-aged mice

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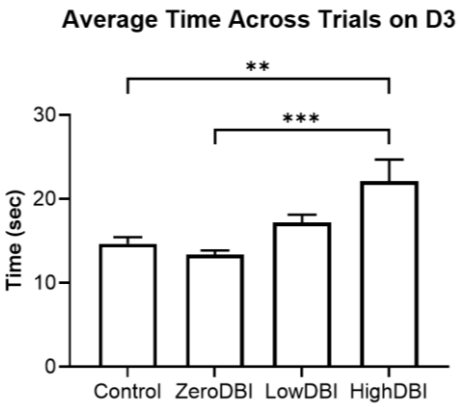
Introduction. Older people are prone to age-related morbidities and are frequently prescribed polypharmacy (concurrent use of 5 medications or more). Polypharmacy and high Drug Burden Index (DBI – cumulative exposure to anticholingergic/sedative drugs) are known to impair central connections related to cognition and some aspects of motor function. However, the effects of polypharmacy and DBI on motor balance and fine co-ordination are unknown.
Aims. In old-aged mice, we aimed to determine the effects of chronic polypharmacy and DBI on motor balance and coordination.
Methods. The balance beam test was performed on old C57BL6 mice (24 months) treated from the age of 12 months with polypharmacy regimens of five therapeutic drugs with zero, low or high DBI or the monotherapies in the high DBI regimen (metoprolol, simvastatin, citalopram, oxybutynin, and oxycodone). Each animal was challenged to cross a 1m beam with 6mm width. Outcomes were the time to complete, proportion of distance that mice dragged across the beam, and the number of slips. Each mouse undertook 3 trials per day for 3 consecutive days.



Results. Preliminary data suggests that high DBI-treated animals, compared to those administered control and zero DBI polypharmacy and monotherapy regimens, took longer to cross the balance beam. The figure shows time to cross beam on Trial 3, Day 3. Reductions in the time to complete the beam were apparent in all animals over successive trials. However, the high DBI-treated animals showed less improvement, consistent with impairments in learning as demonstrated throughout trials over 3 consecutive days. In all animals, dragging distance and slips decreased on day 2 compared to day 1, but the improvements were not a continuous phenomenon through after examination on day 3.
Discussion. Chronic exposure to polypharmacy treatment with high DBI impairs motor balance and learning and improving in motor coordinated functions.

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Strain specific differences between C57Bl/6J and FVB/N mice in the photothrombotic model of stroke.

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Introduction. The photothrombotic model of stroke, which features light induced blood clots, is becoming increasingly popular because of the highly reproducible discrete infarcts. However, despite the increase in popularity, the relationship between the length of light exposure and extent of brain damage has yet to be examined. Furthermore, this model of stroke has been developed in the C57Bl/6J strain of mouse, and yet to be performed in the FVB/N strain.
Aims. To examine the relationship between the length of light exposure and severity of damage, in addition to characterising strain specific differences in stroke outcome between C57Bl/6J and FVB/N mice.
Methods. Under inhaled isoflurane (2-5%) anaesthesia, male C57Bl/6J and FVB/N mice (8 – 10 weeks old) were subjected to a photothrombotic stroke (rose bengal; 10mg/ml ip) followed by 15-(FVB/N: n=16, C57Bl/6J: n=8), 18-(FVB/N: n=14, C57Bl/6J: n=10), or 20-(FVB/N: n=15, C57Bl/6J: n=8) mins light exposure or sham surgery (FVB/N: n=10, C57Bl/6J: n=11). Mice underwent functional testing prior to stroke, and days 1-, 3-, and 7-post-stroke (ps). Infarct volume (thionin staining) and differences in cellular architecture (immunofluorescence) was examined at 7 days ps. Evans blue dye assessed strain differences in blood-brain barrier (BBB) breakdown 4.5h ps.
Results. Increasing the time of light exposure systematically increased infarct volume (C57Bl/6J 15-mins: $9mm^3 \pm 1mm^3$ vs. 20-mins: $14mm^3 \pm 1mm^3$; FVB/N 15-mins: $8mm^3 \pm 1mm^3$ vs. 20-mins: $13mm^3 \pm 1mm^3$; $P<0.001$). However, increased infarct volume did not translate into a worsening of behavioural outcomes. There were strain specific differences in functional outcomes, with C57Bl/6J mice having greater hanging wire deficit than FVB/N mice after 15 mins of light exposure. The opposite was seen in the adhesive removal test. Furthermore, FVB/N mice exhibited greater BBB breakdown compared to C57Bl/6J mice (52%±3% vs. 38%±3% respectively; n = 5, $P<0.01$). Despite this, there was no difference in infarct size, neuronal survival (NeuN), microglial activation (Iba1), T cell recruitment (CD3+) or astrocyte/macrophage infiltration (GFAP, F4/80) between the strains.
Discussion. The volume of damage from the photothombotic stroke can be manipulated by changing the length of light exposure. In addition, FVB/N and C57BL/6J mice exhibit differences in BBB breakdown and subtle variation in functional deficits, which need to be taken into consideration in experimental design.

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A Simple Model of Desensitization for Analyzing Epilepsy-Associated $GABA_A$ Receptor Variants

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Introduction. Developmental and epileptic encephalopathy (DEE) consists of a heterogeneous group of severe epileptic disorders, with many being refractive to treatments. Given the current consensus of epileptogenesis being a consequence of an imbalance between excitatory and inhibitory neurotransmitters, variants that ultimately reduce GABA inhibition through increased receptor desensitization are expected to cause a more severe epilepsy phenotype. Previous research examined receptor desensitization and GABA sensitivity. However, it is ambiguous whether desensitization plays a major role in the severity of phenotype. This may be due to the fact desensitization was determined empirically using deactivation and reactivation kinetics. Thus, we sought to assess the influence of receptor desensitization properties and phenotype by using a range of loss and gain-of-function variants.
Aims. To use a simple but robust equilibrium model of receptor kinetics to determine the desensitization equilibrium and then test it on three variants previously reported to have: 1) increased GABA sensitivity and rate of receptor desensitization; 2) reduced GABA sensitivity and increased rate of receptor desensitization and 3) increased GABA sensitivity without reducing receptor desensitization.
Methods. To investigate this, we used a combination of a simple kinetic model and two-voltage clamp electrophysiology, with concatenated cRNA and *Xenopus Laevis* oocyte, to find the desensitization equilibrium, deactivation, and reactivation rate.
Results. The gamma2R323Q variant had a 1.5 times larger desensitization equilibrium ($p = 0.0003$) and a 9% faster deactivation rate ($p < 0.0001$) than wild-type. In contrast, beta3T287I had a significantly lower desensitization equilibrium constant ($p < 0.0001$) but did not affect the deactivation rate of the receptor. None of the variants affected the reactivation kinetics of the receptor.
Discussion. The results of the desensitization study indicate that the only significant change in receptor desensitization was in the gamma2R323Q variant and the clinical data of patients harbouring this variant suggest that desensitization may be less influential in determining patient phenotype than other channel characteristics.

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Quantifying biased agonism of xanomeline and several muscarinic agonists at the M_4 muscarinic receptor

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Introduction. The M_1 and M_4 muscarinic receptor (mAChR)-preferring agonist, xanomeline is a promising clinical candidate for the treatment of schizophrenia that recently progressed through to Phase III clinical trials (Brannan et al, 2021). Despite this success, very little is known about xanomeline's mode of action at the mAChR family, and in particular, its potential ability to differentially activate downstream signaling pathways (biased agonism) at the M_4 mAChR.
Aim. We aimed to quantify the degree of bias agonism of xanomeline and several other mAChR agonists at the human M_4 mAChR expressed in Chinese Hamster Ovary (CHO) cells.
Methods. Concentration-response curves of several mAChR agonists were generated in 8 distinct signaling assays, including $G\alpha_{i2}$, $G\alpha_{oB}$, $G\alpha_{oA}$ and $G\alpha_{s}$ protein activation assays, as well as β-arrestin-2 recruitment, extracellular signal regulated kinase (ERK) 1/2 phosphorylation, calcium mobilization and exchange protein activated by cAMP (EPAC) activation. Data was fit to the operational model of agonism to derive transduction coefficients and bias factors which describe the difference in activity of an agonist between two pathways, relative to ACh.
Results. Relative to ACh, Xanomeline displayed ~20-fold (18.7 ± 4.38; n=3-7; $P<0.05$) and ~50-fold (54.2 ± 14.4; n=4-7; $P<0.05$) bias towards $G\alpha_{i2}$ activation relative to ERK1/2 phosphorylation and calcium mobilization, respectively. Iperoxo was ~20 biased away from calcium mobilization relative to ERK1/2 phosphorylation, $G\alpha_{i2}$, $G\alpha_{oa}$ and $G\alpha_{ob}$ activation ($P<0.05$) However, in contrast to xanomeline, no other agonists were significantly biased away from ERK1/2 phosphorylation.
Discussion. Our study suggests that xanomeline is biased away from ERK1/2 phosphorylation and calcium mobilization relative to $G\alpha_{i2}$ protein activation. Future research will focus on confirming this pathway bias profile in alternative cellular backgrounds and identifying physiological outcomes in native systems.

Brannan SK et al (2021) *N Engl J Med*. 384(8):717-726

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Developmental changes in brain entry of antiepileptic drugs, valproate and lamotrigine, in a rat model of epilepsy

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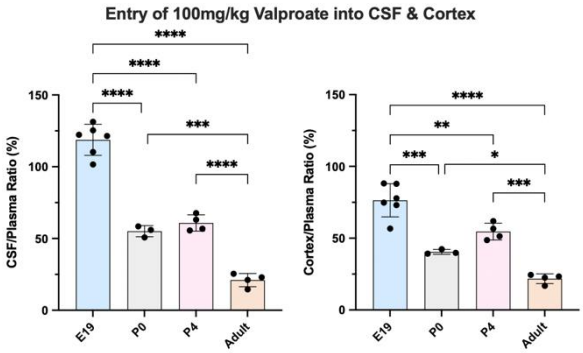
Introduction. *In utero* babies and breastfed newborns may be exposed to maternally administered antiepileptic drugs such as valproate (VPA) and lamotrigine (LTG) when such medications are required to manage maternal seizures. Potential deleterious effects of drugs on the highly sensitive developing brain are not well understood.
Aims. Using an established animal model of absence epilepsy, GAERS (Genetic Absence Epilepsy Rat from Strasbourg), the aims were to 1) to determine transfer of VPA & LTG across placenta; and 2) to investigate entry of both drugs into cerebrospinal fluid (CSF) and brain of pups at different developmental stages.
Methods. GAERS at embryonic day (E) 19, postnatal day (P) 0 & 4 and adults (7 weeks) were administered an ip injection of clinically relevant doses of VPA (30 or 100 mg/kg) or LTG (6 or 20 mg/kg) traced with respective [^{3}H]-labelled drugs. 30 minutes later blood plasma, CSF and brain cortex were collected. In anaesthetised pregnant rats (ip urethane 2.5 g/kg), blood samples from individual pups were serially collected along maternal samples. Radioactivity was measured with liquid scintillation and results expressed as ratios in CSF or cortex over plasma (Mean±SD).
Results. Placental transfer of VPA was around 60%. Both antiepileptic drugs showed age-related decreased entry into the CSF and brain. CSF ratios of VPA decreased from 118.7±10.8% in E19 to 21.1±4.6% in adults and in the brain cortex from 75.6±11.6% to 21.8±3.4% (see Fig). Entry of LTG was higher in E19 compared to P4 (CSF, 79.7±26.4% and 12.6±2.7%; cortex, 77.8±17.7% and 32.7±2.9% respectively).
Discussion. 1) Nearly half of VPA in maternal plasma transferred to the fetus, indicating both, a significant degree of protection provided by the placenta but also transfer into the fetus. 2) Brain entry of both antiepileptic drugs was higher earlier in development, especially for VPA.



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Optimising the safe and effective use of opioids in general practice: Using the nominal group technique for research priority setting

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Introduction: The high prevalence of prescription opioid use and harms in Australia is a cause for concern. With emerging opportunities to conduct primary care research into opioid use, identifying priority research areas from the perspectives of health care professionals and consumer stakeholders, can inform future research efforts to optimise the safe and effective use of prescription opioids in general practice.
Aims: To identify and prioritise research priorities on the safe and effective use of opioids in general practice.
Methods: A group of professional and consumer stakeholders were invited to attend a workshop held in Melbourne, Australia, in May 2021. A nominal group technique was used to explore the key question: "what are the research priorities for the safe and effective use of opioids in general practice?". Research priorities were identified and consolidated through a structured workshop and ranked in priority order using an online survey.
Results: Seventeen experts representing medical, pharmacy, nursing, allied health, policy and consumer disciplines participated in the workshop. A total of 26 priorities emerged from the workshop in three domains: (1) consumer, (2) clinician and practice, and (3) system and policy. We observed relatively evenly distributed votes in each domain; no questions were unanimously voted as a low priority. Consumer characteristics that influence opioid prescribing and outcomes, understanding the outcomes of opioid deprescribing strategies, and the impact of regulatory strategies that aim to restrict opioid supply were ranked highest in consumer, clinician and practice, and system and policy focused domains, respectively.
Discussion: Communication and health literacy, the outcomes of different deprescribing strategies, and the impact of major policy changes were important themes identified across the research questions. These priorities reflect important dimensions of opioid use and specific knowledge gaps within the Australian general practice. The clinically driven and practice-based priorities may inform the direction and focus of future research studies.

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The prevalence of frailty among older adults living with dementia: A systematic review

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Introduction. Frailty is an important geriatric syndrome that increases the risk of poor health outcomes in older adults with chronic conditions such as dementia. However, the prevalence of frailty in older adults living with dementia and its relation to medication use remains unclear.
Aims. The aims of this review were to investigate the prevalence of frailty in older adults living with dementia and explore the differences in medication use according to frailty status.
Methods. A systematic search was performed in Embase, Medline, International Pharmaceutical Abstracts, APA PscyInfo, CINAHL, Scopus and Web of Science from inception to 20 August 2020. Two reviewers independently screened records and conducted quality assessment using the Newcastle-Ottawa Scale
Results. Sixteen articles met the inclusion criteria, with seven studies conducted in acute care settings and nine studies in community-dwelling setting. Five studies recruited people with dementia exclusively and 11 studies were conducted in older populations that included individuals with dementia diagnosis. Frailty was defined using a range of assessment tools. Among studies conducted in acute care setting, the prevalence of frailty ranged from 50.8% to 91.8% compared to studies in the community which reported a prevalence of 24.3% to 98.9%. With respect to medication use, three studies documented medication use according to frailty status but not dementia status. Higher medications use, measured as total number of medications was reported in frail (7.0±4.0(SD)-12.0 ±9.0(SD)) compared to non-frail participants (6.1±3.1(SD)-10.4 ± 3.8(SD)).
Discussion. Current data suggests a wide range of frailty prevalence in individuals with dementia. Future studies should systematically document frailty in adults living with dementia and its impact on medication use.

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Prevalence and risk factors for drug-related problems in people with dementia living in the community: a systematic review and meta-analysis

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Introduction. Dementia is marked by a decline in cognition which is not a normal part of the aging process. People with dementia have a higher risk of experiencing drug related problems (DRPs) due to multimorbidity, polypharmacy and cognitive and functional impairment.
Aims. To conduct a systematic review and meta-analysis to identify the prevalence and risk factors associated with DRPs in people living with dementia in the community.
Methods. Six databases (Embase, Medline, PsycINFO, International Pharmaceutical Abstracts, Scopus and CINAHL) were searched using a combination of keywords and Medical Subject Heading (MESH) terms with four concepts: dementia, older adults, drug-related problems, and community-dwelling. Primary outcomes were adverse drug events (ADEs), adverse drug reactions (ADRs) and medication errors (MEs).
Results. There were 22 studies included: four cross-sectional studies and 18 cohort studies. The number of participants in these studies ranged from 81 to 21795. The pooled prevalence for any ADEs, including ADRs, in people living with dementia was 19.0% (95% CI: 11.6%-27.7%) while the pooled prevalence for specific types of ADEs ranged from 2.6% to 10.2%. Furthermore, the prevalence of MEs ranged from 0.9% to 41.3%. Psychotropic medications, polypharmacy and inappropriate medications contributed to an increased risk of experiencing DRPs while support with medication management was a protective factor.
Discussion. The overall prevalence of DRPs experienced by people with dementia was higher than general community-dwelling older people and lower than hospitalized people with dementia. Awareness that certain medication, patient, and medication management factors are associated with the risk of people with dementia experiencing DRPs may guide clinicians to identify high risk situations and implement suitable mitigation strategies. Further research is needed to explore the effectiveness of deprescribing and other medication management strategies in reducing DRPs in people with dementia living in the community.

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Potentially inappropriate medications in older surgical inpatients

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Introduction. Prescription of potentially inappropriate medications (PIMs) are associated with increased risk of morbidity and mortality in older adults. While several studies have described prescribing patterns in general medical and aged care cohorts, few studies examined prescription of PIMs in older inpatients admitted to surgical services.
Aims. To evaluate the prevalence of PIMs at admission and at discharge for older inpatients of a surgical service.
Methods. This was a prospective cohort study of 137 consecutive patients aged ≥65 years admitted to a tertiary vascular surgery unit. Prevalence of PIMs at admission and discharge was identified using all tables in the 2019 Beers Criteria. Medications with anticholinergic and/or sedative effects were identified using the Drug Burden Index (DBI).
Results. In this cohort (n=137) there were 214 PIMs identified by Beers criteria at admission and 224 PIMs at discharge (p=0.29). At admission 102 (74.5%) patients had at least one PIM identified by Beers criteria compared to 103 (75.2%) patients at discharge (p=0.89). There were 83 prescriptions of DBI-contributing medications at admission compared to 95 prescriptions at discharge (p=0.05). Of the 137 patients, 48 (35.0%) patients were on DBI-contributing medications at admission compared to 53 (38.7%) patients at discharge (p=0.53). The most frequently identified classes of PIMs at both admission and discharge by Beers criteria were diuretics (loop and thiazide), proton pump inhibitors, opioids and antiepileptics (predominantly pregabalin); the most frequent DBI-contributing classes were antiepileptics (pregabalin) and opioids, with selective serotonin reuptake inhibitors being a distant third.
Discussion. PIMs as identified by either Beers criteria or contribution to DBI were prevalent at admission and at discharge. Deprescribing did not appear to occur during a routine surgical admission, with similar rates of PIM prescription at admission and at discharge. There was a trend towards increased new prescriptions of DBI-contributing medications. Further investigation is needed to explore the association between PIMs and adverse outcomes in older surgical inpatients and to determine whether deprescribing interventions can be implemented to reduce PIMs in this population.

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Pharmacogenetics in Papua New Guinea HIV patients beyond *CYP2B6*

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Introduction. Papua New Guinea (PNG) has the highest prevalence of HIV/AIDS in the Pacific. Efavirenz (EFV) shows large variability between patients in terms of plasma concentrations, yet, very little is known about the non-*CYP2B6* genetics (transport and metabolism) in the PNG population and their impact on plasma EFV concentrations (Bordin Andriguetti et al., 2021) We hypothesised that the frequency of genetic variants in *ABCB1, CYP2A6*, *CYP3A4* and *UGT2B7* will be significantly different compared to East Asian, European and African populations.
Aims. To determine the frequency of *ABCB1 (61A>G, 1199G>A, 1236C>T, 2677G>T and 3435C>T), CYP2A6 (48T>G, 5065G>A), CYP3A4 (15389C>T) and UGT2B7 (1306A>G)* SNPs in PNG HIV/AIDS patients receiving efavirenz. To compare allele frequencies between PNG and East Asian, European, African and South Asian populations and to examine the relationship between these SNPs and plasma EFV concentrations.
Methods. Whole blood and plasma were collected from 154 PNG HIV/AIDS patients. EFV plasma concentrations were determined by LCMS/MS. DNA was genotyped by MassArray panel through AGRF. Allele frequencies were compared to other populations by Fisher's exact test. Differences between genotypes in plasma EFV concentrations were determined using nonparametric Kruskal-Wallis test.
Results. Variant allele frequencies of *ABCB1 (1236C>T, 2677G>T and 3435C>T)* and *CYP2A6 (48T>G)* are the highest reported for any population in the world. There is no statistically significant difference (p>0.99, OR 1.01, 95%CI 0.75 to 1.37) between the PNG UGT2B7 (1306A>G) variant allele frequency and that of the African population with the highest variant allele frequency in the world.
Discussion. PNG HIV/AIDS patients exhibit very high frequencies of key SNPs involved in drug transport and metabolism which may have important implications for substrate drugs in this population. These results may also help to better explain plasma variability in EFV concentrations beyond *CYP2B6* alone.

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A novel therapeutic approach for the concurrent treatment of chronic respiratory and cardiovascular disease.

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Introduction. Chronic obstructive pulmonary disease (COPD) is an irreversible disease consisting of a persistent airflow limitation, lung inflammation and oxidative stress, primarily caused by cigarette smoke (CS) exposure. COPD is the 3rd leading cause of death worldwide with 50% of all COPD patients dying from cardiovascular disease (CVD). It is understood that oxidative stress and inflammation orchestrate vascular remodeling and dysfunction. However, there are currently no effective therapeutics available that can treat both the pulmonary and cardiovascular comorbidities of COPD simultaneously.
Aim. To examine whether the antioxidant apocynin may be used to concurrently treat lung inflammation and vascular dysfunction in a mouse model of COPD.
Methods. Male BALB/c mice were exposed to either room air (sham) or CS (9 cigarettes/ day, 5 days/ week) for 8 weeks to induce COPD. Mice were injected once daily with apocynin (5 mg/kg, i.p.) or vehicle (0.01% DMSO diluted in sterile PBS) 1 h prior to the initial CS exposure of the day. Bronchoalveolar lavage fluid was collected to assess lung inflammation. The thoracic aorta was excised and used for myography or immunohistochemistry. Cumulative concentration-response curves to acetylcholine (ACh) and sodium nitroprusside (SNP) were performed, to assess endothelial and smooth muscle dilator responses, respectively.
Results. In sham-exposed mice, ACh caused ~95% relaxation of U46619-precontracted aorta irrespective of apocynin treatment (n=8). CS-exposed mice had significantly impaired aortic relaxant responses to ACh (n=8, ~50 % R_{max}, p<0.0001) which was improved by apocynin treatment (n=8, ~75% R_{max}, p<0.0001). Moreover, apocynin significantly reduced lung inflammation in CS-exposed mice.
Discussion. Apocynin reduces CS-induced lung inflammation and vascular endothelial dysfunction suggesting antioxidant treatment may be a novel means to simultaneously treat both lung and cardiovascular manifestations in COPD.

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ACE2 expression in organotypic airway epithelial cultures and asthmatic airways

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Introduction. The Coronavirus disease 2019 (COVID-19) is an acute respiratory disease caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that may have cardiovascular complications, and long-term systemic impacts. Therapeutic strategies for COVID-19, including repurposing (partially) developed drugs are urgently needed, regardless of the increasingly successful vaccination outcomes.
Aims. We characterised two-dimensional (2D) and three-dimensional models (3D) to establish a physiologically relevant airway epithelial model with potential for investigating SARS-CoV-2 therapeutics.
Methods. Human airway basal epithelial cells maintained in submerged 2D culture were used at low passage to retain the capacity to differentiate into ciliated, club, and goblet cells in both air-liquid interface culture (ALI) and airway organoid cultures, which were then analysed for cell phenotype makers. Airway biopsies from non-asthmatic and asthmatic donors enabled comparative evaluation of the level and distribution of immunoreactive angiotensin-converting enzyme 2 (ACE2).
Results. The ACE2 and transmembrane serine proteinase 2 (TMPRSS2) genes were expressed in ALI and airway organoids at levels similar to those of native (i.e., non-cultured) human bronchial epithelial cells. ACE2 is mainly localised to ciliated and basal epithelial cells in human airway biopsies, ALI, and airway organoids. Neither asthma nor smoking status had consistent marked influence on the expression or distribution of ACE2 in airway biopsies. SARS-CoV-2 infection of ALI cultures did not increase the levels of selected cytokines.
Discussion. Organotypic, and particularly ALI airway cultures are useful and practical tools for investigation of SARS-CoV-2 infection and evaluating the clinical potential of therapeutics and vaccines for COVID-19.

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Anti-Inflammatory Influences of Cystic Fibrosis Drug Ivacaftor on Lung Inflammation

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Introduction. Cystic fibrosis (CF) is a life-limiting multisystem disease caused by a dysfunction in the cystic fibrosis transmembrane conductance regulator protein (CFTR). CF is associated with a myriad of respiratory complications, notably, an increased susceptibility to lung infections and inflammation. Sustained and progressive inflammatory insults lead to continuous airway damage and remodelling, resulting in compromised lung function. Ivacaftor is a small molecule potentiator which targets the defected CFTR channel. Treatment with ivacaftor was shown to significantly improve respiratory function and reduce the incidence of pulmonary exacerbations. The potential therapeutic and prophylactic effects of ivacaftor on CF-related lung inflammation has not yet been previously fully elucidated.
Methods. Female C57BL/6 mice were assigned to one of 3 experimental groups: control, therapeutic and prophylactic. All experimental groups received intratracheal administration of lipopolysaccharide (LPS, 10 ug). Prophylactic treatment involved intraperitoneal (ip) injections of ivacaftor (30mg/kg) once a day for the 4 days prior to LPS challenge. Mice were treated therapeutically with a single ip ivacaftor injection (30mg/kg) directly after LPS challenge. Mice were culled either 24h or 72h post LPS challenge and blood, bronchoalveolar lavage fluid (BALF) and lung tissue samples were collected to determine levels of inflammation through cell and cytokine analysis. Lung histology will be assessed to determine whether ivacaftor reduces structural lung damage caused by inflammation.
Results. Prophylactic ivacaftor treatment significantly increased total inflammatory cells in BALF compared to both the control and therapeutic experimental groups 24h post LPS challenge. Differential cell analysis indicated that macrophages were the cells that mostly comprised the change identified in the infiltrates in prophylactic treatment group compared to control and therapeutic groups 72h post LPS.
Discussion. Ivacaftor results in a change in composition of lung cell infiltrates to inflammatory stimuli. Further analysis of systemic and lung cell profile and function is required to identify how Ivacaftor exerts these effects.

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Targeting Formyl Peptide Receptors as a Novel Approach to Treat Pulmonary Hypertension

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Introduction. Pulmonary hypertension (PH) is a progressive disease with limited treatment options. Elevated mean pulmonary arterial pressure (mPAP) leads to right ventricular (RV) remodelling and failure. Aims. To evaluate the therapeutic potential of a formyl-peptide-receptor (FPR) agonist Compound 17b (Cmpd17b) in models of PH. Methods. C57BL/6J mice were subjected to either hypoxia (10% O_2) with sugen (20mg/kg, sc, weekly in first 3 weeks) or normoxia with vehicle (0.5% carboxymethyl cellulose in 0.9% tween 80 and 0.4% benzyl alcohol in saline, sc) and followed for 28 days. In a separate cohort, mice were administered bleomycin (BLM, 1 mg/kg, oropharyngeal) or saline and followed for 21 days. All mice received daily injections of either vehicle (10% dimethyl sulfoxide in 0.8% tween 80 in saline, ip), Cmpd17b (50 mg/kg, ip) or sildenafil (1.4 mg/kg, ip). At the experimental endpoint, mice were anaesthetised (ketamine/xylazine/atropine, 100/20/1.2 mg/kg, ip), RV systolic pressure (RVSP) and mean arterial pressure (MAP) were measured, and tissues were collected for further analyses. Results. Mice subjected to sugen-hypoxia (SuHx) displayed a moderate yet significant increase in RVSP and Fulton's Index (RV/LV+S, Table), whereas mice subjected to BLM had a markedly lower RVSP and increased lung weight. Cmpd17b or sildenafil did not significantly affect RVSP, but Cmpd17b significantly decreased lung weight and fibrosis. Discussion. Although Cmpd17b had no significant impact on the elevation of RVSP, its efficacy on RV and vascular remodelling remains to be explored.

Results (Mean ± SEM, n)						
	Control	SuHx + vehicle	SuHx + Cmpd17b	SuHx + sildenafil	BLM + vehicle	BLM + Cmpd17b
RVSP	28.3 ± 0.9 (8)	37.3 ± 2.4 (9) **	36.6 ± 1.8 (8)	35.6 ± 1.3 (10)	23.0 ± 1.6 (4)**	24.7 ± 0.5 (4)
MAP	79.3 ± 4.1 (6)	69.9± 3.4 (9)	64.3 ± 3.9(7)	58.3 ± 5.9 (7)	72.6 (2)	68.4 ± 2.5 (4)
RV/LV+S	0.29 ± 0.01 (8)	0.47 ± 0.02 (6) ****	0.46 ± 0.04 (6)	0.48 ± 0.01 (6)	0.28 ± 0.02 (5)	0.31 ± 0.02 (5)
Lung Weight: Tibia Length	7.52 ± 0.28 (5)	10.5 ± 0.41 (5) **	9.78 ± 0.59 (6)	10.1 ± 0.5 (5)	12.1 ± 0.78 (5) ****	9.12 ± 0.5 (5) ##
**p<0.01, ***p<0.001, ****p<0.0001 compared to control, ##p<0.01 compared to BLM + vehicle (One-way ANOVA with Sidak's post hoc test). Right ventricular systolic pressure (RVSP), Sugen-Hypoxia (SuHx), Bleomycin (BLM), Right ventricle (RV), Left ventricle (LV), Septum (S).						

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***In-vitro* bronchodilation to FFA agonists TUG891 and GW8508 is maintained in mouse models of allergic airways disease and/or obesity.**

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Introduction. New bronchodilators are required to overcome the limitations of current therapy with the β_2-adrenoceptor agonist salbutamol (SALB), as its efficacy is reduced in severe asthma. Obesity is a common comorbidity associated with more frequent and severe asthma exacerbations. Free fatty acid receptors 1 and 4 (FFAR1 and FFAR4) are expressed in the airways and under investigation as targets for asthma treatment. Here, we assessed whether bronchodilation to FFA agonists cause is maintained in models of allergic airways disease and/or obesity.

Aim. To test GW9508 (GW, FFA1/4 agonist) and TUG891 (TUG, FFA4) in mouse models induced by house dust mite (HDM)) and/or high fat diet (HFD).

Methods. 6-week-old C57BL/6 naïve female mice were weighed weekly while on normal diet (ND) or HFD for a total of 17 weeks. From weeks 13-17, mice were challenged five times/week with intranasal sterile PBS or HDM, giving four groups of mice (ND/PBS, ND/HDM HFD/PBS, and HFD/HDM) At week 18, mice were euthanized for preparation of precision cut lung slices (PCLS) to measure changes in airway area under phase contract microscopy. Airways were precontracted with 300nM methacholine before concentration-response curves to TUG and GW. Lung sections from separate PCLS were stained with H&E and ABPAS for scoring of airway inflammation and goblet cells.

Results. Weight gain was greater in all HFD mice by week 7 compared to ND ($p<0.05$), while goblet cell scores were similarly increased in ND/HDM and HFD/HDM at week 18. In ND/PBS mice, both FFA agonists had similar efficacy (% maximum relaxation: TUG 84±4, n=9; GW 79±9, n=7) but TUG was more potent (pEC_{50}: TUG 5.3±0.1; GW 4.7±0.2). Both HDM and HDM decreased the potency of TUG, while in the combined model potency was further decreased 10-fold (pEC_{50} in HFD/HDM: TUG 5.3±0.1, n=6) without affecting maximum relaxation. HFD and/or HDM had no effect on GW-mediated relaxation.

Conclusion. Both GW9508 and TUG891 maintained their efficacy in the models, however loss of potency of TUG891 suggests that FFA4-mediated dilation may be compromised in asthma. Further studies are required for comparison with current therapy and validation in human airways to support clinical translation.

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Ebselen negates viral-induced exacerbations of skeletal muscle dysfunction in cigarette smoke-exposed mice.

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Introduction. Chronic obstructive pulmonary disease (COPD) is characterised by progressive and irreversible airflow limitation that is largely attributed to cigarette smoking (CS). Skeletal muscle dysfunction affects up to 40% of people with COPD and is further worsened following an episode of viral-induced acute exacerbation of COPD (AECOPD), which may prolong hospital stay and lead to future readmission. It is becoming clear that increased oxidative stress plays an active role in muscle dysfunction. However, whether increased oxidative stress may be responsible for the further deterioration of muscle dysfunction in AECOPD remains unknown.
Aims. To define the role of oxidative stress in skeletal muscle dysfunction during viral-induced AECOPD.
Methods. Airway inflammation was established in male BALB/c mice by exposure to cigarette smoke (CS) for 8 weeks. Exacerbation was then induced by intranasal inoculation with influenza A virus (IAV, Mem71, $1x10^{4.5}$ PFU). The antioxidant, ebselen (10mg/kg per day; oral gavage) was administered 1 hr prior to the first CS exposure of the day and throughout the IAV infection period. Muscle function and key parameters were assessed on day 3 post-infection, when airway inflammation was at its peak.
Results. Eight weeks of CS exposure (representing stable COPD) resulted in muscle dysfunction as evidenced by a ~20% loss of muscle mass and a ~36% decrease in strength of the tibialis anterior (TA) muscle (p<0.0001 vs sham; n=6). Virus infection of CS-exposed mice resulted in a further ~24% reduction in TA strength, without further loss of muscle mass (p<0.01 vs CS Veh; n=6). Despite the fully preserved muscle mass and strength under stable COPD condition (p<0.01 vs CS Dil; n=6), ebselen administration during viral exacerbation only partially recovered muscle strength (p<0.01 vs CS IAV; n=6), to levels of stable COPD condition.
Discussion. Virus infection further deteriorated muscle strength in CS-exposed mice independent of muscle mass. Inhibition of oxidative stress by ebselen specifically negated the added deterioration of muscle strength during a viral exacerbation, suggesting oxidative stress is an important driver of the worsened skeletal muscle dysfunction in AECOPD.

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Paediatric ADHD medication exposures in Australia: a retrospective study, 2004-2019

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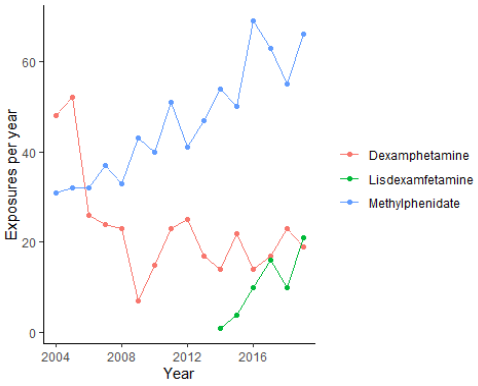
Objective: To describe time trends in attention-deficit hyperactivity disorder (ADHD) medication exposures in children under five years; describe patient demographics, medications involved, exposure reasons, and disposition.
Design: A population-based, retrospective cohort study of calls to Australia's largest Poisons Information Centre. Exposure counts and dispensing-adjusted rates were modelled with Poisson, quasi-Poisson, and negative binomial regression where appropriate.
Setting: Calls to the NSW Poisons Information Centre and dispensings on the Pharmaceutical Benefits Scheme.
Results: There were 1,175 exposures to ADHD psychostimulants, 2004 to 2019; averaging 73 per year. Accidental exposures accounted for 94% of cases. Methylphenidate was most frequently implicated (63%). Thirty-four percent of cases were referred to hospital and a further 21% of calls were made by hospital staff. Exposure counts for all ADHD psychostimulants increased by 2.7% (95%CI = 0.42 to 4.9%) per year; however, this differed by agent. Methylphenidate exposures increased by 5.2% per year (95%CI = 4.3 to 6.1%), lisdexamfetamine increased by 62% per year (95%CI = 48 to 76%), while dexamphetamine exposures decreased by 5.5% per year (95%CI = -9.5 to -1.4%). These trends are reflected in the number of dispensings; however, dispensings increased at a faster rate than exposures. When exposures were expressed as dispensing-adjusted rates, there was a 16% decrease (95%CI = -20 to -13%) per year.



Conclusions: ADHD medication use has increased, associated with an increased number of paediatric poisonings. However, poisonings per script dispensed has decreased. The majority of cases required hospitalisation, indicating the need for further poisoning prevention strategies.

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Aromatic amine metabolomic prediction in developing Ames test predictive models

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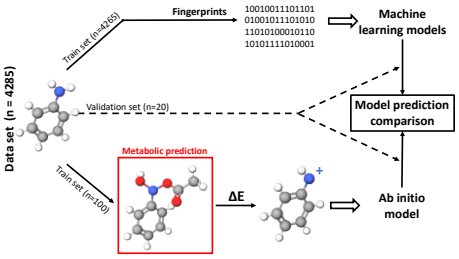

Introduction. Aromatic amines (AAs) are a class of compounds of regulatory concern that includes numerous Ames positive mutagens. Proposed mutagenic mechanisms of action focus on formation of nitrenium ions (R-NH$^+$) that then bind to DNA. Published AA *in silico* predictive models used fingerprints or the energy of formation (ΔE) of the nitrenium ion from the AA but have yet to consider metabolic pathways.
Methods. Key metabolites in the nitrenium ion formation pathway were selected from Biotransformer metabolite prediction of the Brüschweiler and Merlot (2017) data set (n=23). A set of quantum molecular (QM) descriptors and the nitrenium ion energy of formation was calculated for each. Metabolites were then sorted into families based on parent AA. A larger data set (n=4285) of AAs extracted from three publicly available databases was used to generate predictive models based on the MACCS, RDKIT, Morgan, and Mordred 2D descriptors of the AAs.
Results. Metabolic prediction produced 107474 molecules from the 23, of which 196 followed common nitrenium ion formation pathways. Preliminary minimum metabolite ΔE calculations were suitably able to differentiate Ames positive and negative AAs. The process took approximately 45min per AA compared with sub second times for non-QM descriptors. A preliminary non-QM models trained on a subsampled data set (50:50 +ve/-ve n=2000) was developed.
Discussion. This project proposes a QM metabolomic AA Ames predictive model methodology to be compared to non-QM QSAR predictive models. The QM metabolic model provides more mechanistic interpretability however high calculation time means faster but less accurate QM methodologies (HF-3c) are needed for feasible large data application. A successful QM metabolic model could be applied to regulatory AA screening processes providing mechanistically interpretable results.

Brüschweiler and Merlot (2017). Regul. Toxicol. Pharmacol. 88: 214-226.

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Biochemical changes in ram spermatozoa after in vitro exposure to common herbal supplements

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Introduction: Complementary and alternative medicines are used frequently, despite a lack of regulation and research into their safety and efficacy. Supplements marketed towards 'boosting fertility' are popular, but there is little research into their effects on spermatozoa. Previous studies looking at supplementation of sperm focus on motility rather than biochemical changes, and there is little research regarding potential toxicity in sperm.
Aim: The aim of this study was to determine whether in vitro incubation with maca root induced biochemical changes in ram spermatozoa.
Method: The function and metabolism of ram spermatozoa, incubated in media containing *Lepidium meyenii* (maca root) were investigated. At 0.5, 3 and 6 hours post-exposure, computer-assisted sperm analysis (CASA) and flow cytometry were used to assess motility, viability, acrosome reaction, membrane lipid disorder, mitochondrial superoxide production, intracellular reactive oxygen species (ROS) and DNA fragmentation.
Results: Treatment with maca induced acrosome reaction in treated cells (2.02%± 0.39 n=3 $p < 0.001$), but there was no difference in viability (73.3%±1.49 n=180 $p > 0.05$). There was an increase in mitochondrial superoxide production across all treatments and time points, and maca promoted membrane lipid disorder across all treatments (17.28%±0.85 n= 54 $p < 0.001$).
Discussion: The promotion of premature acrosome reaction in absence of an ovum may impact fertility of the male. It is posited that this may be driven by the oestrogenic activity of maca. Oestrogenic activity may also contribute to the higher membrane fluidity of treated sperm, as oestrogen can induce capacitation-like changes. Despite reported antioxidant activity of maca, the increase in mitochondrial superoxide production suggests increased oxidative stress in the presence of maca. Contrary to popular belief that these products enhance fertility, this research indicates that they should be used with caution due to possible negative effects on sperm, and lack of stringent safety data.

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Towards a molecular docking approach for structure-based virtual screening of endocrine disrupting chemicals acting at the retinoic acid receptor.

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Introduction. The retinoid signalling pathway plays a vital and diverse role in the regulation of embryonic development, reproduction, and general hormonal processes. Accordingly, a 2020 review by European Commission scientists (Grignard et al.) has advocated for retinoid pathways to be included in OECD endocrine disruptor testing guidelines. The Tiered Protocol for Endocrine Disruption (Schug et al.) has proposed in-silico techniques, such as QSAR and molecular docking, as a frontline New Approach Methodology to reduce animal use in toxicological assessment.
Aims. To demonstrate the utility of molecular docking as an in-silico screening method for (a) the physicochemical characterisation of retinoic acid receptor binding and (b) detection of retinoid-based endocrine disrupting chemicals.
Methods. A crystal structure of the ligand binding domain of retinoic acid receptor gamma (RARγ) bound with all-trans retinoic acid (atRA) was extracted from the Protein Data Bank (PDB ID: 2LBD). AutoDockTools v1.5.6 was used to prepare the atRA and RARγ structures and precompute atom-specific interaction energies within the binding site. 10 docking runs were performed using a Lamarckian genetic algorithm in AutoDock v4.2.6.
Results. Self-docking of atRA in the RARγ binding site returned 10 poses all within 0.98 Å root mean square deviation (RMSD) of each other, with the lowest energy pose returning a RMSD of 0.53 Å from the reference 2LBD crystal atRA conformation. Analysing this pose within the RARγ binding site reveals predominantly hydrophobic interactions surrounding the β-ionone ring of atRA with a specific cluster of polar residues that anchor the carboxylate tail of atRA.
Discussion. Initial findings demonstrate the ability for molecular docking to reliably reproduce native atRA binding conformations. Elucidation of the physicochemical parameters governing atRA-RAR binding, notably hydrophobicity and H-bonding, will enable continuing work on the development of a custom scoring function to be used for the cross-docking and high-throughput virtual screening of potentially endocrine disrupting chemicals in the RAR binding site.

Grignard et al. (2020) Repro. Toxicol. 93: 250-258. Schug et al. (2013) Green Chem. 15: 181-198.

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Flavonoid-statin interactions and the possible significance of OATP transport, CYP450 metabolism and mevalonate synthesis.

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Introduction. Supplements containing large doses of flavonoids have the potential to lead to statin-adverse events by inhibiting hepatic organic anion transporting polypeptide (OATP) and cytochrome 450 (CYP450). The inhibition of mevalonate production by statins is a hypothetical pathway for statin myotoxicity, since flavonoids can also inhibit mevalonate synthesis, co-administration of flavonoids with statins may lead to pharmacodynamic interactions.
Aims. To establish a liver cell line using HepG2 cells to model statin-flavonoid interactions and identify if OATP, CYP450 or mevalonate inhibition play a role in this.
Methods. Pre-treatment of HepG2 cells with rifampicin to induce CYP450 activity was validated with paracetamol CYP450 dependent toxicity. The fluorescent OATP substrate pyranine was used to quantify OATP1B1 activity in HepG2 cells via spectrophotometry in the presence or absence of flavonoids. Cells will also be exposed to combinations of statins and flavonoids in the presence or absence of mevalonate.
Results. Paracetamol produced concentration-dependent toxicity in HepG2 cells and rifampicin pre-treatment significantly increased this, suggesting induction of CYP450. Simvastatin, fluvastatin and rosuvastatin all produced concentration dependent toxicity within HepG2 culture. There was significant concentration dependent uptake of pyranine in the absence of flavonoids, suggesting functional OATP in HepG2 cells.
Discussion. This preliminary data confirms the presence of OATP1B1 and CYP450 activity within HepG2 cells and validated these methodologies for testing the significance of these pathways in statins. Statins on their own produce toxicity in HepG2 culture and modulation of their toxicity by flavonoids in the presence or absence of mevalonate can now be undertaken.

Turner RM *et al* (2019). J Clin Med 9: 22
Yang W *et al* (2017). Xenobiotica 47: 86-92.

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Potentiating effect of tadalafil and sildenafil on vasodilation of the porcine superior vesical artery

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Introduction: Ischaemia of the bladder may play a role in the aetiology of bladder dysfunction. Recent literature suggests that phosphodiesterase (PDE) 5 inhibitors may have beneficial effects in relieving ischaemia and ameliorating symptoms, via vasodilation of the bladder vasculature (Andersson, et al. 2017).
Aims: The aim of this study was to determine whether the clinically used PDE-5 inhibitors tadalafil and sildenafil can potentiate nitric oxide-mediated vasodilation of the porcine superior vesical artery (SVA) model.
Methods: Porcine SVA were obtained from a local abattoir. Circular sections, with endothelium intact, were mounted in organ baths, containing oxygenated physiological solution (37˚C). Arterial rings were vasoconstricted using noradrenaline (NA), in the absence and presence of nitric oxide (NO) synthase inhibitor L-NNA. Vasodilatory responses to endogenous endothelium-dependent NO release (muscarinic-receptor agonist carbachol) and exogenous endothelium-independent NO release (SNAP, SIN-1 and sodium nitroprusside) were also obtained, in the absence and presence of PDE inhibitors tadalafil, sildenafil and papaverine. Statistical differences were determined using two-tailed paired student's t-tests, with $P<0.05$ considered significant.
Results: In the presence of the NO-synthase inhibitor L-NNA, vasoconstriction responses to SVA via NA were significantly ($P<0.05$, n=6) enhanced by 45%, without change to the potency of NA. Additionally, endogenous NO evoked vasodilatory responses were abolished in the presence of L-NNA. All PDE inhibitors, papaverine, tadalafil and sildenafil significantly ($P<0.001$, $P<0.01$ and $P<0.01$) depressed NA evoked vasoconstrictions (58%, 26% and 35%). Potency of NO donors evoked vasodilation (sodium nitroprusside, SIN-1 and SNAP) was significantly potentiated by all inhibitors, with no change to maximal response.
Conclusion: Endothelium-independent vasodilation of the porcine SVA is potentiated by the clinically used PDE-5 inhibitors tadalafil and sildenafil. This suggests that the isoenzyme PDE-5 is present in this tissue, and that tadalafil and sildenafil act on endothelium-independent NO-mediated vasodilatory pathways, which may contribute to their beneficial effects in ameliorating bladder symptoms.
Andersson, KE; Boedtkjer, D; Forman, A; Therapeutic Advances in Urology, 2017, 11-27.

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Distinctive expression of mas-related G protein-coupled receptor-X2 in inflammatory bowel disease and acute diverticular disease; does it renew the potential involvement of neuropeptides in colonic inflammation?

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Introduction. Mas-related G protein-coupled receptor-X2 (MRGPRX2), originally found on mast cells, has been shown to respond to several stimuli, including neuropeptide substance P (SP) causing mast cell degranulation as well as inducing inflammatory responses. Although SP and neurokinin NK1 receptors are strongly implicated in the pathophysiology of inflammatory bowel disease, the role of MRGPRX2 and its association with SP in colonic inflammation is underexplored. In this study, we aimed to localise MRGPRX2-immunoreactivity (IR) in the human colon, and to compare the expression level of MRGPRX2 in the colon of ulcerative colitis (UC), Crohn's disease (CD) or acute diverticular disease (DD) with age-, gender- and region-matched controls.
Methods. Full thickness of colonic tissue was fixed in Zamboni's fixative, embedded and 5 µm sections were incubated with anti-MRGPRX2 antibody (cat# ab167125 and cat# ab237047, Abcam) followed by fluorescent secondary antibodies and DAB. The slides were scanned by Aperio and quantitatively analysed by QuPath.
Results. MRGPRX2-IR was present on immune cells within mucosal lamina propria and the submucosal layer, where a few of MRGPRX2-IR cells co-localised with tryptase positive mast cells and IBA-1 positive macrophages. However, the identities of most MRGPRX2-IR-containing cells remain to be determined. MRGPRX2-IR was downregulated in both mucosa and submucosa layers of UC ($P < 0.05$, Wilcoxon paired t-test) and the mucosa of DD ($P < 0.01$). In contrast, an increased MRGPRX2-IR was seen in both mucosa and submucosa of CD ($P < 0.05$). Moderate MRGPRX2-IR was localised to myenteric ganglionic cell bodies, where it was co-expressed with cells positive for Hu-C/D (nerve cell body marker), SP, VAChT and NOS. Downregulated MRGPRX2-IR in myenteric ganglia was seen in DD ($P < 0.01$) but not in UC and CD.
Conclusions. MRGPRX2 was primarily expressed in immune cells in the mucosa and submucosa of the human colon with significant upregulation in CD and downregulation in UC and DD. The contrast in UC and CD findings were unexpected, and further research should be guaranteed to address underlying reasons. The decreased MRGPRX2-IR in myenteric ganglia of DD may be relevant to reduced contractile responses in this disease. Since SP can activate MRGPRX2, SP may mediate proinflammatory actions via stimulating MRGPRX2, previously ascribed to NK1 receptors.

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Multiple treatments with cyclophosphamide cause physiological changes to the murine urinary bladder.

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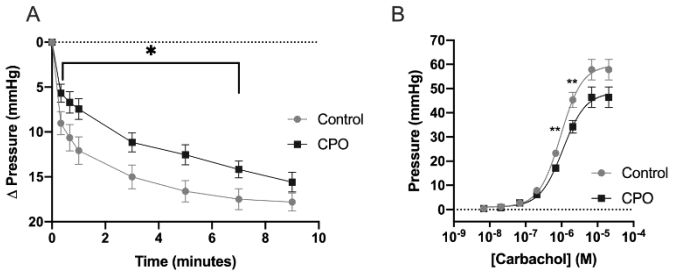
Introduction. Cyclophosphamide (CPO) is a chemotherapeutic agent used for treating a variety of cancers and autoimmune disorders. However, patients often experience urological adverse effects such as frequency, urgency, nocturia, incontinence and cystitis after treatment. The mechanisms by which CPO causes these adverse effects is relatively unknown, and this lack of knowledge pertaining to the functional and urological adverse effects of CPO are a major limiting factor of the drug treatment.



Aims. Investigate the effects of multiple doses of CPO on bladder physiology in an ex-vivo whole bladder preparation.

Methods. Female C57BL/6J mice (12-13 weeks) were used. Mice were euthanised, bladders were isolated and placed in 37°C Krebs-bicarbonate solution and aerated with carbogen (95% O2 and 5% CO2) gas. Whole bladder preparations were performed as previously described by our group (West, E.G. et al, 2018). Spontaneous activity, bladder compliance and accommodation, detrusor contractility and nerve-evoked contractile responses were measured.

Results. A significantly reduced fall in intravesical pressure was observed in bladders from CPO treated mice during accommodation when compared to controls (Figure 1A, $P<0.05$, ANOVA multiple comparisons with Tukey correction). Potency of muscarinic receptor agonist carbachol was unchanged between CPO and sham treated groups. However, contractile activity was decreased at concentrations above 10^{-6}M in CPO treated bladders, with significant differences at 10^{-6}M ($P<0.01$, one-tail, unpaired Student's t-test) and $3x10^{-6}$M ($P<0.01$, one-tail, unpaired Student's t-test).

Discussion. CPO treatment significantly affected accommodation of bladders and decreased pressure responses to the muscarinic receptor agonist carbachol. This data highlights the implications of multiple exposures to CPO on physiological function of the bladder, which may contribute to the urotoxic effects seen in patients.

References. West, E.G, et al, 2018. J. Pharmacol. Exp. Ther. 366: 282-290.