Poster abstracts

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Raising awareness and early detection of undiagnosed atrial fibrillation through a systematic population-based screening program in Tasmania

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Introduction. Atrial fibrillation (AF) is a significant contributor to stroke and can be asymptomatic. Systematic population-based screening can promote AF awareness and identify people with undiagnosed AF in the community. However, information on systematic population-based screening for AF is lacking in Australia.

Aims. To assess the prevalence of previously undiagnosed AF and to promote AF awareness in Tasmania.

Methods. People aged ≥65 years with no history of AF were recruited through community events and media advertisements. Screening sessions for previously undiagnosed AF were conducted using the Microlife WatchBP Home-A blood pressure monitor at public events, health expos, shopping centres and clubs across Tasmania. Participants with positive screening results were referred to their general practitioners to confirm the presence of AF. These participants were followed up to determine the outcomes of the screening. At the screening venues, AF educational campaigns such as health talks and distribution of AF resource materials were provided to the public.

Results. A total of 1,704 eligible participants were screened at 79 sessions across Tasmania. Of these, 50 (2.9%) had a positive screening result. After a follow-up of these participants, the presence of AF was investigated in 47 (94%), and the device correctly identified AF in 22 (46.8%) and produced 25 (53.2%) false-positive results. Among those with confirmed AF, 6 (27.3%) had a history of AF but were not aware of the diagnosis, and 16 (72.7%) were identified to have previously undiagnosed AF, with a prevalence of 0.9% (95% CI, 0.58 to 1.52). Oral anticoagulation therapy (OAC) was initiated in 12 (87.5%) of those with a CHA_2DS_2VA score of ≥2. Also, 50% of all the participants became aware of AF through participation in the screening program and educational campaign.

Conclusion. A systematic population-based screening for AF identified 0.9% of older people (≥65 years) with previously undiagnosed AF in Tasmania. Although the performance of the Microlife WatchBP Home-A device was suboptimal under the study conditions, our findings indicate the possible benefits of systematic population-based screening and awareness raising for AF in the community.

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Correlating functional studies of human bitter taste receptors (T2Rs) in humans and mice.

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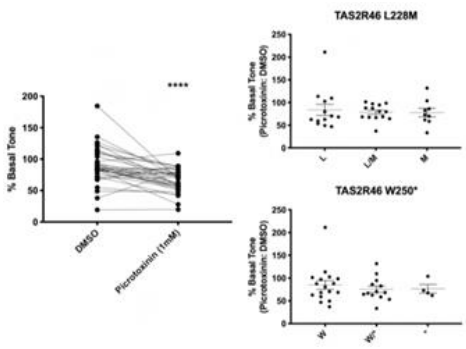

Introduction. We have reported the expression of T2Rs in the heart – activation of these receptors with picrotoxinin (a ligand for T2R46) in explanted human atrial trabeculae causes decreased contractility. Highly penetrant polymorphisms in T2R46 are non-functional in cell-based assays, but such a functional association in explanted cardiac tissue remains to be confirmed (Figure 1). As a corollary, we are also developing a humanised mouse model, where adeno-associated viruses deliver human T2R46 to cardiomyocytes in vivo. The lack of homology between rodent and human T2Rs provides a unique opportunity unambiguously determine the role of human T2Rs and polymorphisms within the heart.

Methods. Explanted right atrial appendages were obtained from patients at the Prince Charles Hospital. Dissected trabeculae were mounted and electrically paced before ligand-mediated changes in tissue contractility were recorded in response to picrotoxinin (1 mmol/L final). Hearts were isolated from mice injected with a cardiac-specific adeno-associated virus construct that expresses both a T2R and eGFP, and perfused in the Langendorff model. Cardiac parameters were recorded during infusion of increasing concentrations of picrotoxinin.

Results. In human cardiac tissue, the addition of picrotoxinin resulted in diminished cardiac contractility (Figure 1) and there does not appear to be a strong association with established T2R46 SNPs. Control experiments indicate that picrotoxinin does not bind/activate mouse T2Rs and does not cause cardio-depression in uninfected, isolated mouse hearts. Testing virally expressed human receptors are the focus of current experiments.

Discussion. T2Rs are expressed within the cardiovascular system and are thought to play a role in regulating normal cardiovascular physiology i.e. contractility and vascular tone. Studying human receptors and their genetic variants requires the establishment of models that can unambiguously delineate their function.

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Pharmacological plasma cell depletion with Bortezomib does not attenuate angiotensin II-induced hypertension.

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Introduction. B cell-depletion is known to blunt experimental hypertension (Chan et al, 2015), which suggests that B cells play a significant role in elevations in blood pressure (BP). B cells can differentiate into antibody secreting cells (ASCs) such as plasmablasts and plasma cells (Drummond et al, 2019), however, whether antibody production is a primary role for B cells in hypertension remains unclear.

Aims. To determine the effect of pharmacological depletion of ASCs on experimental hypertension.

Methods. Ten week-old C57BL6/J mice were randomly assigned to receive either angiotensin II (0.7mg/kg/day; *s.c.*) or vehicle (0.5% NaCl, 0.1% acetic acid) via osmotic minipump for 28 days. To deplete ASCs, the proteasome inhibitor, bortezomib (0.75mg/kg) or its vehicle (0.1% DMSO) were administered (*i.v.*) 3 days prior to minipump insertion, and then twice weekly thereafter. Weekly BP measurements were recorded by tail-cuff plethysmography. After 28 days of treatment, ASC abundance was measured in spleen and bone marrow. All experiments were performed blinded to treatment groups.

Results. Bortezomib treatment reduced the frequency of splenic plasmablasts ($CD138^{hi}Sca\text{-}1^{+}Blimp\text{-}1^{+}B220^{+}$) and plasma cells ($CD138^{hi}Sca\text{-}1^{+}Blimp\text{-}1^{+}B220^{-}$) by 76% (vehicle vs bortezomib: 0.025 ± 0.006% vs 0.006 ± 0.001%, n=9-11) and 70% (vehicle vs bortezomib: 0.030 ± 0.007% vs 0.009 ± 0.003%, n=9-11), respectively, compared to vehicle-treated mice. Bone marrow plasma cells, but not plasmablasts, were also reduced by 75% (vehicle vs bortezomib: 0.008 ± 0.002% vs 0.002 ± 0.001%, n=9-11) in bortezomib-treated mice. However, bortezomib has no effect on angiotensin II induced hypertension (vehicle vs bortezomib: 172 ± 7 vs 182 ± 4 mmHg, n=9-11).

Discussion. Pharmacological depletion of ASC did not ameliorate angiotensin II-induced hypertension, which suggests B cells may act via an alternate mechanism to promote experimental hypertension.

Chan et al (2015) Hypertension 66:1023-1033.
Drummond et al (2019) Nat Rev Immunology 19(8):517–32

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The pro-resolving lipid mediator lipoxin A_4 protects against inflammation in diabetic cardiomyopathy

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Introduction. Failure to resolve inflammation may contribute to the progression of diabetic cardiomyopathy. We have previously demonstrated that the pro-resolving lipid mediator lipoxin A_4 (LXA_4) attenuates the development and progression of diabetes-induced atherosclerosis, but its impact on diabetic hearts has not been fully explored.

Aim. To test the hypothesis that LXA_4 could attenuate cardiac inflammation in diabetic mice.

Methods. 6-week-old male $ApoE^{-/-}$ mice were followed for 16wks after streptozotocin (55mg/kg/day i.p. for 5 days)-induced diabetes or vehicle control. Mice were randomly allocated to receive either LXA_4 (5µg/kg) or vehicle (0.02% ethanol) via i.p. injections twice/week for the final 6wks. At the end of the study, mice were culled with an overdose of Sodium Pentobarbital (100mg/kg), organs harvested for *ex-vivo* analysis.

Results. Diabetic mice displayed elevated HbA1c levels, retarded body weight gain, increased infiltration of macrophages in the myocardium and elevated expression of M1-like macrophage marker (Table). Interestingly, administration with LXA_4 significantly decreased the expression of M1-macrophage maker m*S100A9* and inflammatory marker m*Il-1β*. The macrophages content was no longer evident in the diabetic mice treated with LXA_4.

	Non-diabetic mice		Diabetic mice	
	Vehicle	**LXA_4**	**Vehicle**	**LXA_4**
Body weight (g)	32.06±1.78 (n=17)	30.63±2.50 (n=8)	25.71±1.38**** (n=7)	25.57±2.51**** (n=7)
HbA1c (%)	4.55±0.58 (n=15)	4.56±0.52 (n=15)	11.81±1.10**** (n=7)	11.14±1.40**** (n=7)
Macrophage content (No./0.43mm²)	11.73±4.04 (n=13)	12.39±4.31 (n=12)	18.18±7.82** (n=9)	14.08±3.36 (n=9)
m*S100A9* (fold increase)	1.00±1.92 (n=17)	0.49±0.31(n=7)	4.78±6.40** (n=7)	0.54±0.32# (n=7)
m*Il-1β* (fold increase)	1.00±0.95 (n=17)	0.92±0.37 (n=7)	1.59±1.34 (n=7)	0.42±0.13# (n=7)

**p<0.001, ****p<0.0001 vs non-diabetic + vehicle; #p<0.05 vs diabetic + vehicle, (2-way ANOVA, Fisher's post-hoc for multiple comparisons).

Conclusion. LXA_4 may reduce inflammation by promoting the resolution of inflammation in the diabetic heart, thus supporting the development of an LXA_4 based therapy to improve the outcome for patients with diabetic heart diseases.

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Rivaroxaban for people with stage 4 chronic kidney disease and atrial fibrillation

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Background. Patients with stage 4 Chronic Kidney Disease (CKD) and atrial fibrillation (AF) are at high risk of both stroke from their AF and bleeding from anticoagulation for stroke prevention.(Kimachi et al, 2017;Weir et al, 2020)) Until recently rivaroxaban was contraindicated in Australia for patients with AF and stage 4 CKD (GFR 15-30ml/min). In June 2020 the Australian Product Information (PI) was amended to allow rivaroxaban use in these patients. Rivaroxaban has advantages over warfarin in this population due to slower decline in renal function, and no clear association with vascular calcification.(Coleman et al, 2019; Yao et al, 2017; Zhang et al, 2019) It is unclear what proportion of stage 4 CKD patients with AF at our hospital were prescribed anticoagulation of any form before the PI change. This is important because this indicates another opportunity to prevent strokes in this cohort.

Aims: To determine what proportion of patients with stage 4 CKD and AF were anticoagulated prior to the rivaroxaban PI change.

Method. A retrospective observational audit was conducted in a random sample of inpatients with AF and stage 4 CKD between June-December 2019, to determine what anticoagulation was prescribed.

Results. To date thirteen patients were audited. 12/13 were prescribed oral anticoagulation (7/13 warfarin, 4/13 apixaban 2.5mg twice daily, 0/13 rivaroxaban, 1/13 dabigatran 110mg twice daily).

Discussion. Our results indicate that patients with stage 4 CKD and AF were prescribed anticoagulation at a high rate before the rivaroxaban PI change. Results also suggest these patients are mostly prescribed either warfarin or apixaban. There is concern that apixaban 2.5mg twice daily is a less effective treatment dose for AF (Alexander et al, 2016). Switching these patients to rivaroxaban may help reduce the risks of warfarin related vascular calcification and renal disease progression and allow use of a more effective dose of anticoagulation compared to apixaban 2.5mg twice daily. Further studies are warranted to examine prescriber views/attitudes to rivaroxaban use in this high-risk patient group.

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Anandamide-induced vasodilatation in normotensive and hypertensive rats

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Introduction. Anandamide, an endogenous agonist of cannabinoid CB_1 receptors and transient receptor potential vanilloid 1 (TRPV1) channels, can inhibit vasoconstriction and decrease blood pressure by modulating sympathetic and sensory neurotransmission. The effect of anandamide action on vascular tone in hypertension is unclear.

Aims. To examine the effect of anandamide on arterial tone in 16-week-old male normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) and ascertain its mechanism(s) of action *in vivo*.

Methods. In anaesthetised rats (2% isoflurane mixed with O_2; spontaneous inhalation via nose cone), intravital microscopy was used to investigate mesenteric arterial diameter. Anandamide concentration-response curves were generated in U46619-constricted (300 nmol/L) arteries in the absence and presence of i) the CGRP receptor antagonist, BIBN 4096 (1 µmol/L); ii) the fatty acid amide hydrolase (FAAH) inhibitor, URB937 (100 nmol/L) to inhibit anandamide degradation; and iii) capsaicin (10 µmol/L) to desensitise sensory nerves. Similar experimental protocols were performed in isolated mesenteric arteries via wire myography.

Results. Anandamide caused concentration-dependent relaxation in arteries from both rat groups. Maximum relaxation (Rmax) was greater in WKY rats than in SHR (89±9 vs. 47±5%, n=7 and 6, respectively; $P<0.05$). URB937 enhanced anandamide-mediated vasodilatation in SHR only (Rmax, 89±6%, n=5; $P<0.05$). Capsaicin abolished anandamide-induced relaxation in both groups (Rmax, 1±6 and 11±8%, respectively, n=5; $P<0.05$) while BIBN 4096 had no effect. In contrast, *in vitro* anandamide relaxed pre-constricted mesenteric arteries isolated from WKY rats and SHR with similar potency and efficacy (pEC_{50}, 6.22±0.04 vs. 6.31±0.05, respectively) and URB937 had no appreciable effect on anandamide-mediated relaxation. Capsaicin inhibited anandamide-mediated relaxation *in vitro* and its inhibitory effect was more marked in SHR than WKY rats (63-fold vs. 23-fold decrease in pEC_{50}, respectively; $P<0.05$).

Discussion. Compared to WKY rats, anandamide-mediated relaxation was impaired in SHR *in vivo*, possibly due to a higher level of expression and/or activity of FAAH in SHR arteries. Although the inhibitory effects of capsaicin initially suggest the involvement of sensory nerve activation in anandamide-mediated vasodilatation, the absence of inhibition following BIBN 4096 treatment suggests that sensory nerve-derived CGRP is not involved in anandamide-mediated vasodilatation *in vivo*. In contrast to arteries in the intact circulation, FAAH activity may be limited in isolated arteries.

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Pravastatin targeting of myometrial artery endothelium-dependent vasodilation in preeclampsia

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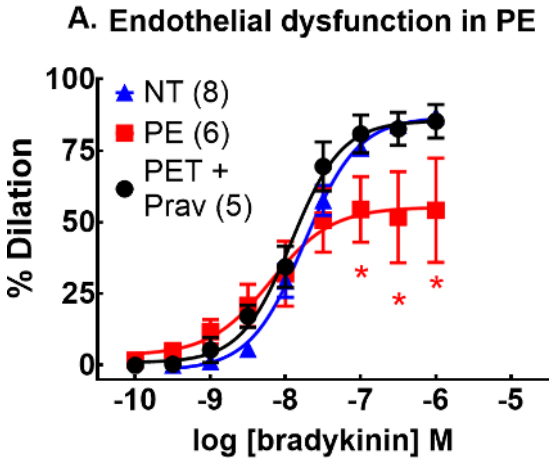
Introduction: Preeclampsia (PE) causes significant maternal and fetal morbidity and mortality. Maternal vascular endothelial dysfunction contributes to PE resulting in systemic organ dysfunction. Effective therap emain to be identified.

Aims: This study aims to investigate the effect of *in vitro* pravastatin on uterine microvascular endothelial function in PE.

Methods: Myometrial radial arterioles from caesarean-section normotensive (NT) and PE patients were incubated with pravastatin (2mM/6h) *in vitro*. Electron microscopy, immunohistochemistry and pressure myography with pharmacological intervention characterized vessel structure and function.



Results: Overall caveolae density/µm is reduced 24% from 5.5±0.1 in NT (n=4, P<0.05) to 4.2±0.01 (n=4, P<0.05) in PE vessels, a further 31% to 1.9±0.8 (n=4, P<0.05) in PE vessels following treatment with pravastatin. Confocal immunohistochemistry showed reduced caveolin-1 expression in PE arterioles relative to NT, expression being reduced further in PE vessels following treatment with pravastatin. Functionally, PE vessels exhibited decreased vasodilator NO and IKCa activity, relaxation being predominantly S and BK_{Ca} mediated. Pravastatin incubation restored endothelium-dependent relaxation in PE samples to NT levels (Figure), enhancing the NO and IK components of relaxation.

Discussion: This data suggests treatment of preeclamptic vessels with pravastatin can be associated with improved vessel function as well as modulation of caveolae form and distribution.

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Deletion of orphan GPCR, GPR37L1, alters autonomic control of cardiovascular homeostasis in mice

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Introduction. GPR37L1 is an orphan G protein-coupled receptor with a reported role in maintaining blood pressure (Min et al, 2010), though a mechanistic explanation for this is currently unclear. Since GPR37L1 is expressed highly in the brain and not in the heart or kidney (Coleman et al, 2018), we propose GPR37L1 may alter autonomic control of the cardiovascular system.

Aims. This series of experiments was designed to identify whether GPR37L1 is necessary for normal autonomic system control of cardiovascular homeostasis.

Methods. Blood pressure, heart rate (HR) and locomotor activity were recorded by radiotelemetry in C57BL/6J and GPR37L1$^{-/-}$ mice of both sexes. Auto- and cross-spectral power analysis of mean arterial pressure (MAP) and HR was used to decipher cardiovascular autonomic contribution. Pharmacological ganglionic blockade (pentolinium) was used to determine sympathetic vasomotor tone. Cardiovascular reactivity to stress was determined by subjecting mice to acute physical stress tests (dirty cage swap, restraint, palatable food presentation) while telemetered.

Results. GPR37L1$^{-/-}$ genotype had a statistically significant positive effect on HR across both sexes (genotype effect p=0.0002, two-way ANOVA). Both sexes of GPR37L1$^{-/-}$ mice exhibited attenuated depressor responses to ganglionic blockade, indicating reduced sympathetic vasomotor tone. There was a reduction in the night-time HR power spectra of female GPR37L1$^{-/-}$ mice within a frequency band correlated with vagal drive. Interestingly, female GPR37L1$^{-/-}$ mice exhibited an attenuation of cardiovascular reactivity to aversive, but not appetitive, environmental stimuli.

Discussion. Together, these results suggest that loss of GPR37L1 impairs vagal drive of HR, reduces sympathetic vasomotor tone, and differentially affects male and female cardiovascular responses to stress.

Min et al. (2010) Biochem Bioph Res Co 393:55-60
Coleman et al. (2018) Biol Sex Differ 9:14

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Psychometric properties of self-reported medication adherence tools in cardiovascular disease: a systematic review

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Introduction. Many self-reported medication adherence (SRMA) tools have been developed and validated in patients with cardiovascular disease (CVD); however, it is not known which SRMA tool is most suitable for measuring medication adherence in patients with CVD.

Aims. This review aimed to evaluate the psychometric properties of SRMA tools to measure medication adherence in adults with CVD.

Methods. An electronic search was conducted in nine databases including PubMed, MEDLINE, CINAHL, ProQuest Health and Medicine, Cochrane Library, PsychInfo, Scopus, Embase, and Web of Science. Studies that have reported at least one of the psychometric properties for a SRMA tool in patients with CVD were included. COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN) checklist was employed for assessing the methodological quality of the studies.

Results. The review included 74 studies and identified 38 separate SRMA tools. These tools were classified into three groups based on their medication adherence domains; group-1 tools had items with information specific to the extent of adherence, group-2 tools had items dealing with reasons for non-adherence, and group-3 tools asked about both adherence domains. The Voils extent of non-adherence tool from group-1, MASES, MASES-R, and SEAMS tools from group-2, and ARMS from group-3, had the most robust psychometric properties in their group. The most frequently assessed tool was MMAS-8 from group-3 with good psychometric properties; however, there was moderate evidence of insufficient results in its internal consistency.

Discussion. No study has evaluated all nine psychometric properties in a single study. Tools including the Voils extent of non-adherence tool, ARMS, MASES, MASES-R, and SEAMS showed robust psychometric properties to measure medication adherence in adults with CVD. Researchers and health care providers need to carefully consider the type of adherence phase, and the comprehensiveness of the SRMA tools, in addition to the adequacy of their psychometric properties.

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The nitroxyl donor Angeli's salt circumvents nitric oxide resistance in the insulin-resistant diabetic myocardium

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Introduction. Diabetes increases mortality risk due to cardiovascular complications, which are partially driven by impairments in nitric oxide (NO•) signalling at the level of tissue responsiveness, known as NO• resistance.

Aims. To investigate whether diabetes promotes, and nitroxyl (HNO) circumvents, NO• resistance in the myocardium.

Methods. At 8 weeks of age, male Sprague-Dawley rats were fed a high-fat diet and 2 weeks later received low-dose streptozotocin (2x35 mg/kg ip, over 2 consecutive days). At 22 weeks of age, we assessed responses to the NO• donor diethylamine NONOate (DEA/NO) and the HNO donor Angeli's salt in Langendorff-perfused hearts. Responses to insulin were also examined in Langendorff-perfused hearts to assess cardiac insulin sensitivity. Data are expressed as change from baseline (Δ) and were analysed by Student's unpaired t-test. *P<0.05 vs non-diabetic (ND) hearts.

	DEA/NO (10^{-5} M)		**Angeli's salt (10^{-5} M)**		**Insulin (33.3 IU)**	
	ND (n=8)	**Diabetic** (n=8)	**ND** (n=8)	**Diabetic** (n=9)	**ND** (n=7)	**Diabetic** (n=9)
ΔLVDP (mmHg)	5.7±0.7	2.3±0.6*	4.1±0.7	10.2±1.4*	20.3±2.7	6.3±1.9*
ΔLVEDP (mmHg)	2.6±0.6	1.1±0.2*	1.8±0.3	1.6±0.2	1.5±0.2	0.8±0.2*
ΔLV+dP/dt (mmHg/s)	147±19	96±18*	121±11	183±19*	371±45	83±17*
ΔLV-dP/dt (mmHg/s)	-133±8	-78±7*	-102±13	-153±14*	-339±46	-82±15*
ΔCoronary flow (mL/min)	7.0±0.7	4.6±0.9*	6.5±0.7	7.7±1.1	1.8±0.3	1.0±0.1*
ΔHeart rate (bpm)	14.5±2.3	21.2±1.3*	9.6±1.0	11.8±1.2	4.8±0.8	4.0±1.0

Results. Myocardial insulin resistance was evident in diabetic hearts, as demonstrated by blunted inotropic, lusitropic and coronary vasodilator responses to insulin. In response to DEA/NO, inotropic, lusitropic and coronary vasodilator responses were impaired in diabetic hearts, whereas responses to Angeli's salt were enhanced or preserved.

Discussion. These findings demonstrate for the first time that the HNO donor Angeli's salt circumvents NO• resistance in the diabetic insulin-resistant myocardium, highlighting the therapeutic potential of HNO donors to treat acute diabetes-associated impairments in cardiac function.

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Spatial reference memory impairment is augmented in hypertensive mice following stroke

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Introduction. Cognitive impairment is an aging-related disorder that can arise as a result of cardiovascular pathology or cerebrovascular injury. Considering the aging of our population, the incidence of cognitive impairment is thus expected to rise. Hypertension is a major modifiable risk factor for stroke and cognitive impairment, but it is unclear whether it may worsen post-stroke cognitive outcomes.

Aims. This study aimed to determine the effect of hypertension on post-stroke cognitive outcomes.

Methods. C57BL/6J mice (n=80) were randomly assigned to receive chronic infusion of either saline or angiotensin II (0.7 mg/kg/day s.c.) via osmotic minipump. Systolic blood pressure was measured weekly by tail-cuff. Seven days after minipump implantation, mice underwent either sham or photothrombotic stroke surgery targeting the prefrontal cortex, an area that is important for spatial reference memory. A separate cohort of mice underwent daily testing using the Barnes maze test from days 22 to 26. Results. Angiotensin II increased systolic blood pressure (saline, 118±1 mmHg vs. Ang II 149±2 mmHg; $P<0.05$) but this was not affected by stroke (Ang II + sham, 151±4 mmHg vs. Ang II + stroke 148±2 mmHg). In the Barnes maze, hypertensive mice that received stroke surgery took longer to enter the escape hole when compared to other groups (escape latency: Ang II + stroke 142.2 s vs. Ang II + sham 124.6s vs. saline + stroke 109.9 s vs. saline + sham 105.2 s), suggesting that they have poorer spatial reference memory.

Discussion. These findings indicate that the combination of hypertension and stroke resulted in more severe spatial reference memory impairment and brain injury than either insult alone.

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Efficacy and safety of metaraminol infusions in critical care: A systematic review and meta-analysis

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Introduction. Noradrenaline is the preferred first-line vasopressor according to guidelines in the treatment of shock in critical care. However, there has been increasing use of metaraminol infusions as a first-line agent in Australasia and Europe in rates up to 42%. The lack of guidance of metaraminol use in published guidelines has not limited its use in the critical care population.

Aims. To assess the efficacy and safety of metaraminol infusions in comparison to other vasopressors in critically ill patients with shock with regard to hospital mortality, effect on haemodynamics (mean arterial pressure or systolic blood pressure), duration of vasopressor use, and adverse events.

Methods. A systematic review and meta-analysis of controlled trials and observational studies was conducted. Eight electronic databases and nine trial registers were searched from inception to 18 August 2020.

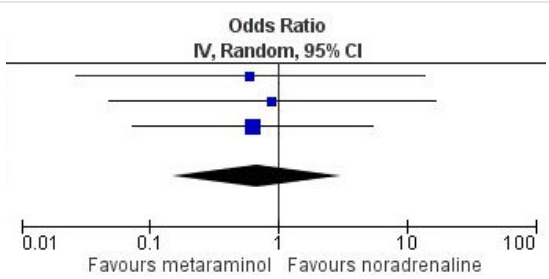

Fig 1a: Hospital mortality forest plot

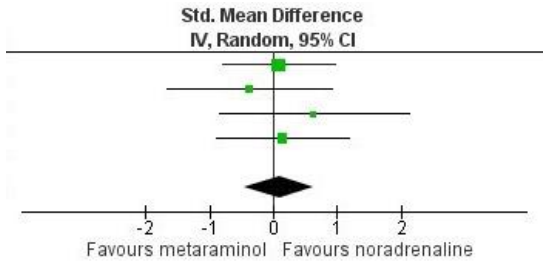

Fig 1b: Blood pressure forest plot

Results. Out of 1387 eligible articles, three observational studies and one controlled trial were included involving a total of 54 patients. Study patients had different types of shock including septic (n=28), cardiogenic (n=21), hypovolemic (n=3) and neurogenic shock (n=2). All studies compared metaraminol to noradrenaline and were of low quality and high risk of bias. There was no difference in hospital mortality between metaraminol and noradrenaline groups (OR 0.68, 95% CI 0.15 to 3.09; p=0.62). There was no difference in the effect on blood pressure between metaraminol and noradrenaline (standardised mean difference 0.09, 95% CI -0.46 – 0.64; p=0.76). None of the studies reported the duration of vasopressor use or adverse drug events such as extravasation or tissue injury.

Discussion. There is limited and low-quality efficacy and safety data to support the use of metaraminol over other vasopressors in critically ill patients with shock.

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The association between menopausal status and remission in rheumatoid arthritis patients receiving disease modifying antirheumatic drugs

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Introduction. Rheumatoid arthritis (RA) is an auto-immune inflammatory disease and is more prevalent in females than males with a ratio of 4:1 at younger ages (<50 years old) and 2:1 at older ages (>60 years old). It has been suggested that alteration in the balance of sex hormones is associated with the development of RA.

Aims. To examine the association between baseline menopausal status and remission likelihood in RA patients treated with tocilizumab (TCZ) and/or conventional synthetic disease modifying antirheumatic drugs (csDMARDs).

Methods. Data were pooled from 5 phase III clinical trials where participants with RA were treated with TCZ and/or csDMARD. Available data included documented baseline menopausal status (pre vs post), age, sex hormone supplement use, weight, race, number of previous DMARDs and baseline disease scores. Remission criteria were set according to the simplified and clinical disease activity index (SDAI and CDAI respectively), and 28-joint disease activity score (DAS28). The association between menopausal status and the time of first remission was assessed via Cox proportional analysis.

Results. The analysis included data from a total of 4,473 female patients treated with TCZ and/or csDMARDs, of which 3028 (68%) were post-menopausal and 1445(32%) pre-menopausal and 232 (7.7%), 284 (19.7%) were using sex hormone supplements, respectively. In the pooled analysis, pre-menopausal status was associated with significantly higher SDAI remission compared to post-menopausal status on univariable (HR 1.21[95%CI 1.08-1.36], P=0.002) and adjusted (HR 1.87 [1.004-3.48, P =0.04] analysis. Similar associations were observed using CDAI and DAS28-ESR remission. Sex hormone use was associated with significantly higher SDAI remission on univariable (HR 1.26[1.07-1.49, P= 0.005]) and adjusted (HR 1.20 [1.01-1.42, P =0.038]) analysis. The association of female reproductive status and sex hormone use with remission were independent of the type of RA therapy (interaction P>0.05).

Conclusion. Premenopausal reproductive status and sex hormone use were independently associated with more frequent remission in female RA patients, regardless of the type of DMARDs used.

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Chronic polypharmacy and increasing Drug Burden Index (DBI) impair cognitive function in aged mice

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Introduction. Ageing, polypharmacy (≥5 medications) and increasing DBI (anticholinergic and sedative medication exposure) are associated with impaired cognitive function. Preclinical models can assess causation and mechanisms

Aims. We investigated whether chronic polypharmacy or monotherapy, with increasing DBI and/or cessation (deprescribing), affected learning and/or memory in ageing mice.

Methods. 12-month-old male C57BL/6 mice received either control diet or study drug(s) at therapeutic doses. The three polypharmacy diets had zero DBI (metoprolol, simvastatin, omeprazole, paracetamol, irbesartan), low DBI (metoprolol, simvastatin, omeprazole, paracetamol, citalopram) and high DBI (metoprolol, simvastatin, citalopram, oxycodone, oxybutynin). Individual drugs from the high DBI regimen were tested as monotherapies. At 21-months, animals were randomly stratified to continue treatment or have it gradually deprescribed. Barnes Maze learning occurred over 4 days, with an introduction to the target hole before trial 1 (only day 1), followed by 4 trials (all 4 days), followed by short-term memory test (ST; day 5) and long-term memory test (LT; day 10) at 12-, 21- and 24-months.

Results. Preliminary analysis showed High-DBI polypharmacy mice (n=23-12; p<0.05) reduced time exploring the target hole at ST with a treatment-age interaction effect showing high-DBI decreased exploration time of target hole at ST and increased at LT with increasing age, compared to control (n=24-29; p<0.05). At 24-months high-DBI and low-DBI showed impaired learning with longer time and distance travelled before arriving at the target hole on day 1 trial 1, while deprescribed high-DBI (n=16) and low-DBI (n=17) performed similar to control.

Discussion. Drug treatment with polypharmacy regimens with DBI>0 increased learning time, and impaired short-term memory. Preclinical results suggest polypharmacy impacts cognition, and further analysis will explore the role of each monotherapy vs polypharmacy.

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How are antibiotics prescribed for open reduction internal fixation procedures?

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Introduction. Surgical antibiotic prophylaxis (SAP) is a common indication for antimicrobial use in Australia, however inappropriate use can contribute to antimicrobial resistance and increase costs. New SAP recommendations in the Therapeutic Guidelines (published April 2019) suggests the use of single dose prophylaxis for internal fixation.

Aim. The aim of this study was to determine how antibiotics are prescribed for patients undergoing open reduction internal fixation (ORIF) of upper and lower limbs at Northern Health following guideline update.

Methods. A retrospective audit was conducted for patients who underwent ORIF of closed fractures between July and December 2019. Medical records were reviewed for antibiotic choice, dose, route and time of administration and duration of prophylaxis.

Results. 209 patients were included in this study. Pre-operative antibiotic administration was documented in 96% of patients. Whilst cefazolin 2g was commonly administered pre-operatively as per guideline recommendations (94% of cases), the majority of patients (79%) received post-operative antibiotics. An extended duration of prophylaxis (>24 hours) was observed in 11% of cases.

Discussion. Variability exists as to whether antibiotics are administered post-operatively, with a need to determine the factors that contribute to an extended duration of prophylaxis. Our findings suggest that guideline amendment, in isolation, does not reflect a change in practice and further research is required to understand what influences prescribing in this setting.

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Cultural Perceptions of Gout in East Asia

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Introduction. Management of gout is suboptimal worldwide, including in East Asia. Cultural perceptions and ethnic-specific factors (e.g. Caucasian stigmatisation; Maori views of stoicism) have been shown to contribute to the poor management of gout in non-East Asian countries. As of yet, no studies have explored perceptions of gout in East Asia.

Aims. To investigate the perceptions of healthcare providers (HCPs) and patients with gout in East Asia to identify barriers to optimal gout management.

Methods. Participants included 8 HCPs and 2 patients with gout from East Asia (China, Taiwan, South Korea and Japan). Semi-structured interviews were conducted and transcribed verbatim. Transcripts were inductively analysed for themes. Data collection ceased once thematic saturation was reached.

Results. HCPs, particularly rheumatologists, reported that delayed or inadequate management of gout was the result of: 1) Patients often considering gout to be a minor illness that did not require medical attention; 2) Clinicians in primary care not being well educated on management strategies and only providing short-term symptomatic treatment. Selection of urate-lowering therapy was influenced by concerns of toxicity, specifically allopurinol hypersensitivity syndrome – a reaction more common in people of East Asian ancestry. Consequently, Taiwanese rheumatologists reported using benzbromarone or febuxostat as the first-line urate-lowering therapy. HCPs and patients alike perceived that there was no negative stigma or sense of shame associated with a diagnosis of gout. Patients also reported that gout was viewed as a minor illness and believed gout to be secondary to their pre-existing renal impairment.

Discussion. Unlike Western cultures, in East Asian communities gout is not associated with negative stigma; rather it is considered by patients to be a minor disease. This perception impedes optimal management and timely medical assistance. Treatment approaches selected by HCPs, particularly in Taiwan, were influenced by reported increased risk of toxicity to allopurinol and association to ethnic genetic predisposition.

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Bayesian approach shows lack of difference between Aboriginal and Caucasian kidney transplant recipients in mycophenolic acid and tacrolimus pharmacokinetics

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Introduction. Aboriginal Australians experience worse patient and graft outcomes following kidney transplantation compared with Caucasian Australians.

Aim. To determine if immunosuppressant pharmacokinetics can explain outcome differences.

Methods. 32 Aboriginals and 14 Caucasians who were >3 months post-transplant and receiving mycophenolate mofetil and tacrolimus underwent a 4 sample PK study. Empirical Bayes estimates (EBEs) of mycophenolic acid (unbound) and tacrolimus (whole blood) clearance were obtained using previously developed PK models (Metz 2018, Storset 2014).

Results. For both drugs, there was negligible difference in median clearance and variability of clearance EBEs between Aboriginal and Caucasian kidney transplant recipients.

Discussion. The Bayesian method leveraged existing knowledge about PK of two immunosuppressants to ask a specific question about race-associated differences in PK. The lack of any evidence for a difference indicates that a target concentration intervention approach to dose individualization (NextDose www.nextdose.org) can use existing PK priors without consideration of Aboriginal race.

Metz, D., N. Holford, J. Kausman, N. Cranswick, A. Walker and F. Ierino (2018). "Preliminary results of the ADOPT trial. Total and unbound mycophenolic acid concentration changes before and after kidney transplantation. " Nephrology 23(54): 1.

Storset, E., N. Holford, S. Hennig, T. K. Bergmann, S. Bergan, S. Bremer, A. Asberg, K. Midtvedt and C. E. Staatz (2014). "Improved prediction of tacrolimus concentrations early after kidney transplantation using theory-based pharmacokinetic modelling." Br J Clin Pharmacol 78(3): 509-523.

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Development and validation of a Liquid Chromatography tandem Mass Spectrometry (LCMSMS) assay investigating pharmacokinetics (PK) of phosphatidylethanol.

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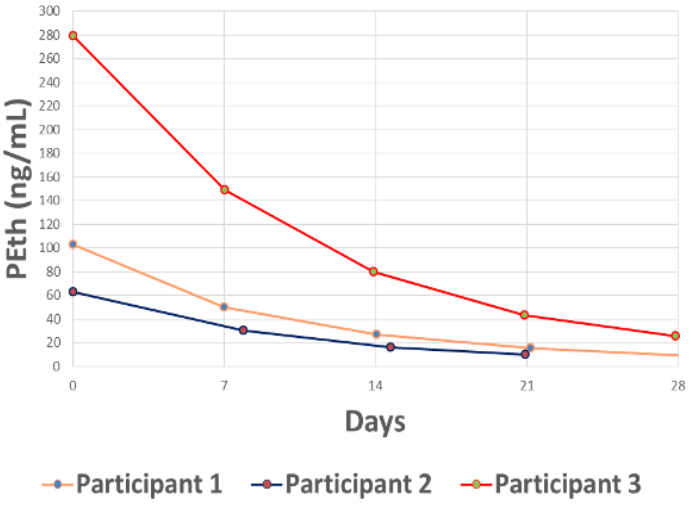
Introduction. Phosphatidylethanol (PEth) has generated interest as a biomarker for ethanol consumption because it is uniquely formed, incorporated and accumulated in red cell membranes by the action of phospholipase D on membrane phosphatidylcholine with ethanol.

Aims. To develop and validate an LCMSMS assay and investigate PK of PEth by monitoring concentrations in saline-washed red cells from participants who keep contemporaneous diaries of ethanol intake.

Methods. PEth was extracted from saline-washed red cells with isopropanol and injected onto a Waters XEVO TQS triple quadrupole LCMSMS. International method validation guidelines were implemented.

Results. Method validation demonstrated linearity between LLOQ of 8ng/mL and ULOQ of 2093ng/mL of washed red cells, with satisfactory inaccuracy, imprecision and signal-to-noise for the LLOQ. Interday, intraday and instrument imprecision was less than 6% over three control concentrations with accuracy greater than 92.9%. Use of a deuterated PEth-d5 internal standard compensated for matrix effects.



Discussion. Ethics approval was obtained to recruit participants in three groups – A) teetotallers, B) drinkers keeping an ethanol diary, and C) drinkers consenting to abstain from ethanol for 4 weeks. Lack of any signal from Group A participants demonstrated specificity of the assay against false positives. Elimination profile from 3 participants in Group C is shown above – demonstrating approximate half-life of 7days with first order kinetics. Preliminary results from Group B demonstrate wide inter-individual PEth concentrations when evaluated against ethanol consumption suggesting need for further investigation of patient covariates for this marker.

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Applications of using Drug Monitoring for Ivacaftor and Tezacaftor Treatment Response in Cystic Fibrosis

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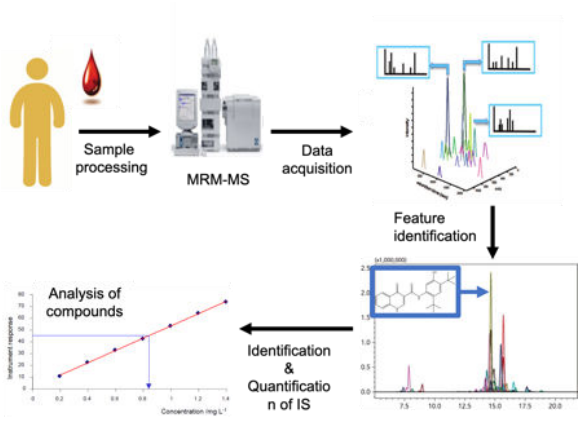
Background: Ivacaftor-tezacaftor is a new breakthrough cystic fibrosis (CF) drug combination that directly modulate the activity and trafficking of the defective CF transmembrane conductance regulator protein (CFTR) underlying the CF disease state. We report the first therapeutic drug monitoring assay for these drugs and their application in the clinic.

Methods: A rapid and precise novel method for the quantification of ivacaftor, its metabolites and tezacaftor in human plasma was developed and validated using multiple reaction monitoring mass spectrometry (MRM/MS).

Results: The MRM/MS analytical method was validated at a concentration range from 0.0025 µg/mL to 1 µg/mL for ivacaftor, ivacaftor-M1, ivacaftor-M6 and tezacaftor in human plasma. The method displayed good accuracy (90.62 -94.51%) and reproducibility (99.91-100%) including at low concentrations 0.01 µg/mL. The reported method can accurately quantify ivacaftor, ivacaftor-M1, ivacaftor-M6 and tezacaftor at low concentrations in human plasma. The assay was successfully utilised in CF patients with severe liver impairment, pharmacokinetic/pharmacodynamic population analysis and quantification studies in animal models.



Conclusion: We have established a cost-efficient and timely method for measuring ivacaftor, its metabolites and tezacaftor in human plasma and tissue samples suitable for high-throughput applications in the hospital settings, clinical trials or in fundamental research.

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A step forward in patient safety - understanding barriers to correct intravenous medication administrations

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Introduction. Medication and procedural errors occur in approximately 75% of intravenous (IV) drug administrations. Relatively few studies have explored nurses' perspectives on factors contributing to, and strategies to prevent, errors.

Aims. To identify common medication and procedural IV errors reported to a voluntary incident reporting system and to explore nurses' perspectives on the factors contributing to, and strategies to mitigate, IV errors.

Methods. IV drug administration incidents reported between January 2015 and May 2020 were reviewed. Medication and/or procedural errors were categorised using the Australian Commission on Safety and Quality in Healthcare's medicine incident classification system. A snowballing approach was used to recruit nurses to participate in interviews about their views on IV medication errors. Emergent themes were identified from interview transcripts.

Results. Of all reported IV medication administration incidents (n=706) 86.1% and 37.2% contained at least one medication and procedural error, respectively. Wrong IV rate was the most frequent medication error type (29%), and failure in double checking and signing procedures the most frequently reported procedural error (19%). Nurses from critical care (n=9) and general wards (n=19) reported that (i) workarounds, developed as a result of time constraints and workload, (ii) lack of education/experience and access to appropriate resources and (iii) difficulties in interpreting policies and procedures, are all contributors to the occurrence of IV drug administration errors. Regular, proactive, case-based education was a commonly identified strategy to prevent IV errors. Development and revision of IV drug administration policies with nurse consultation and amendments to existing models of care to include team-based (e.g. paired) nursing allocations to better support increasing patient acuity were also reported as potential strategies.

Discussion. Delivery of practical training and education on IV infusion rates (e.g. smart pump use and rate calculations) along with improved access to resources (e.g. policies) are potential strategies to minimise wrong IV rate errors arising from lack of experience. Changes to models of care were also suggested to maintain patient safety and minimise errors related to high nurse workloads.

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Predictive performance of population pharmacokinetic models for tacrolimus in lung transplant recipients

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Introduction. Bayesian forecasting software may assist in optimising therapeutic drug monitoring for tacrolimus. However, the most appropriate population pharmacokinetic (popPK) model to be utilised in software to predict tacrolimus exposure in lung transplant (LTX) recipients remains unclear.

Aims. To evaluate and compare the predictive performance of popPK models in post-operative LTX patients. To identify factors which influence the predictive performance.

Methods. Retrospective data from adult LTX patients administered tacrolimus were used to evaluate the performance of 17 published popPK models to predict serum tacrolimus concentrations *a priori* (no observed concentrations included) or with Bayesian forecasting (using concentration data). Predictive performance was determined using relative bias (rBias, bias) and relative root mean squared error (rRMSE, precision). Models were considered clinically acceptable if rBias was between -20% and 20%, and the 95% confidence intervals included zero. The influence of gender, weight, cystic fibrosis (CF), azole therapy and diabetes mellitus status on model performance was assessed with multiple linear regression.

Results. Data from 41 patients (35 non-CF, 6 CF; 1514 concentrations) were used to evaluate 17 tacrolimus popPK models. No models had a satisfactory *a priori* rBias (-111.9 - -46.36). Only the model by Monchaud et al. was clinically acceptable with Bayesian forecasting (rBias -1.82%, CI -3.95 – 0.29; rRMSE 8.85%). Azole therapy was the only covariate with significant influence on the rBias and rRMSE of this model. The incorporation of azole therapy appeared to improve the accuracy of Bayesian forecasting with this model by 8.9% ($p < 0.01$).

Discussion. The model by Monchaud et al. developed exclusively from LTX recipients is suitable to guide tacrolimus dosing in LTX patients. However, a least one tacrolimus concentration is required to ensure accurate predictions.

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Adverse drug reactions presenting to acute medical admissions: a pilot prevalence study

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Introduction. Adverse drug reactions (ADRs), defined by the Edwards and Aronson (2000) definition, accounted for 6.5% of all unscheduled hospital admissions in a 2002 observational study in Liverpool and Merseyside. The authors estimated the projected cost to NHS England was £466 million and 72% of ADRs recorded were potentially avoidable. A retrospective study of ADR-related admissions, defined by ICD-10 code, to English hospitals between 1998 and 2005 reported an admission rate of 0.5%, suggesting under-reporting may be a significant factor in the difference with prospective observational studies.

Aims. To determine the incidence of ADR-related admissions in the University Hospital, Llandough, over a 3 week period, in order to estimate the point-prevalence of ADR-related acute admissions in Wales, compared with our historical coding rate of approximately 0.5%.

Methods. An observational-prospective service audit to describe the point-prevalence of ADR-related admissions to University Hospital Llandough Medical Emergency Assessment Unit over 3 weeks in March 2019. Anonymized participant data was collected from the paper case-notes of unscheduled admissions. ADRs were determined by a consultant-grade clinical pharmacologist.

Results. Thirty-four cases met the inclusion criteria. 32% of the admissions were caused by or contributed to by an ADR (95% confidence interval ($CI_{95\%}$) 0.17-0.51), compared with our historical coding rate of 0.5%, $p = 0.01$. 19% of admissions were directly caused by an ADR ($CI_{95\%}$ 0.068 to 0.35).

Discussion. The prevalence of ADR-related hospital admissions in Llandough hospital was greater than that historically reported by clinical coding. Further, detailed, studies are warranted to improve the identification of ADR-related admissions as a step towards reducing their prevalence.

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Application of physiologically-based pharmacokinetic modelling to understand real-world outcomes in patients receiving imatinib for chronic myeloid leukaemia

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Introduction. There is large variability in imatinib outcomes in the treatment of chronic myeloid leukaemia (CML). Many patients experience severe adverse drug reactions (ADRs) and some never achieve Early Molecular Response (EMR), an important predictor of achieving stable deep molecular response and therefore treatment-free remission.

Aim. To use physiologically-based pharmacokinetic (PBPK) modelling and simulation to predict imatinib steady-state (ss) exposure in patients with CML to investigate variability in outcomes observed.

Methods. A previously validated imatinib PBPK model (Simcyp Simulator v18, Certara) was used to predict imatinib AUC_{ss}, $C_{min,ss}$ and $C_{max,ss}$ for patients from a real-world retrospective observational study. Differences in imatinib exposure were evaluated in patients with different clinical outcomes, (1) EMR achievement (n=45) and (2) occurrence of grade ≥ 3 imatinib-related ADR (n=68), using the Kruskal-Wallis rank sum test. Sensitivity analyses explored the influence of patient characteristics and drug interactions on imatinib exposure.

Results. The patient cohort was 59% male, 74% European ancestry with a median age of 56 years. The majority (71%) had been prescribed at least 1 medication with the potential to interact with imatinib (52% CYP3A4 substrate, 8% CYP3A4 inhibitor, 6% P-gp inhibitor, 7% CYP2C8 inhibitor, 2% CYP3A4 inducer). Simulated imatinib exposure was significantly higher in patients who achieved EMR compared to patients who did not (geometric mean AUC_{ss} 51 vs. 43 µg*h/mL, P<0.05; $C_{min,ss}$ 1.1 vs. 0.9 µg/mL, P<0.05; $C_{max,ss}$ 3.4 vs. 2.8 µg/mL, P<0.05). Patients who experienced grade ≥ 3 ADR had a significantly higher simulated imatinib exposure compared to patients who did not (AUC_{ss} 56 vs. 46 µg*h/mL, P<0.05; $C_{min,ss}$ 1.23 vs. 1.00 µg/mL, P<0.05; $C_{max,ss}$ 3.73 vs. 2.96 µg/mL, P<0.05). The PBPK simulations identified a range of patient (sex, age, total body weight, abundance of hepatic CYP2C8 and CYP3A4, alpha-1-acid glycoprotein concentrations, liver and kidney function) and medication-related factors (dose, concomitantly administered CYP2C8 modulators) that could contribute to the inter-individual variability in imatinib plasma concentration.

Discussion. Relationships between imatinib plasma exposure, EMR achievement and occurrence of severe ADRs support the rationale for therapeutic drug monitoring to guide imatinib dosing to achieve optimal outcomes in CML patients.

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VTE prophylaxis in orthopaedic surgery – Patient individualised or 'one-dose-fits-all'?

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Introduction: Total hip arthroplasty (THA) and total knee arthroplasty (TKA) are one of the highest risks factors for venous thromboembolism (VTE). Therefore, appropriate prophylaxis is imperative. Aspirin for VTE prophylaxis post-THA/TKA is controversial with differing recommendations in international guidelines. It is thought aspirin may not be appropriate for higher risk patients, including those at risk of aspirin resistance: the elderly (≥65 years), obese (body mass index (BMI) ≥30 mg/kg^2), and those with diabetes mellitus or dyslipidaemia.

Aims: To investigate the influence of major risk factors on the selection of VTE prophylaxis after elective THA/TKA.

Methods: A retrospective multisite cohort study between October 2017 and September 2018 of six hospitals in Queensland, Australia. Analysis of VTE prophylaxis (aspirin, rivaroxaban and low-molecular weight heparins (LMWH)) with patient and surgical factors were conducted using Pearson's chi-square.

Risk factors for aspirin resistance	Drug			p-value
	Aspirin	Rivaroxaban	LMWH	
Obese (n=578)	45.3%	42.6%	12.1%	p=0.425
Elderly (n=576)	40.1%	49.0%**	10.9%	p<0.001
Diabetes (n=180)	35.6%	44.4%*	19.4%	p=0.002
Dyslipidemia (n=323)	46.1%	39.9%	13.9%	p=0.76

Results: A total of 1,011 patients (43.1% THA, 56.9% TKA) were included with a mean (SD) BMI of 32.1 (±7.0) kg/m^2, age of 65.9 (±11.0) years and 43.6% males. Discharge prophylaxis was prescribed in 94.4% of patients where aspirin was the most common (42.1%). Compared to rivaroxaban, patients were less likely to be prescribed aspirin if they had diabetes (p=0.002) or age ≥65 (p<0.001). No other major risk factors for aspirin resistance were related to the drug prescribed for discharge VTE prophylaxis.

Discussion: Drug selection did not seem to be impacted by the presence of risk factors for aspirin resistance apart from diabetes and age ≥65 years. The uncertainty of aspirin's efficacy in higher risk patients, in conjunction with its substantial use in patients at risk of resistance is of concern. Further research is required to investigate aspirin resistance post-THA/TKA and the clinical outcomes of resistance.

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A common allosteric mechanism for stabilising agonist ligand binding at GPCRs

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Allosteric modulators represent a novel approach of targeting G protein coupled receptors (GPCRs). Allosteric compounds bind to topographically distinct sites from the highly-conserved endogenous orthosteric pocket, and are capable of modulating both the affinity and efficacy of orthosteric ligands and may have their own intrinsic efficacy[1]. Despite this, there is little information as to how the allosteric modulators stabilise the high affinity active state of the GPCR-G protein ternary complex. This is in part owing to the limitations of attempting to pharmacologically characterise these interactions in recombinant whole cell assays. These cell-based assays are limited by the inability to control the stoichiometry of the interacting partners (receptor & G protein), and the transient nature of the ternary complex owing to the dynamic nature of the GPCR activation cycle and freely available nucleotides (GDP & GTP) to facilitate these transitions. In order to overcome these issues, we reconstituted the M_2 muscarinic acetylcholine receptor (M_2 mAChR) into high density lipoproteins (rHDLs), otherwise known as nanodiscs. Nanodiscs provide a reductionist platform, whereby the relative ratio of GPCR and G protein can be controlled thus enabling the system to be pushed to the low-affinity (GPCR alone) or high-affinity (GPCR-G protein ternary complex) state[2]. Using this approach, we show that allosteric modulators promote the ability of the G protein to stabilise the high affinity active state. Furthermore, whilst allosteric modulators and G proteins both promote the high affinity state in an analogous manner, the allosteric modulator is not able to promote further increases in orthosteric ligand affinity when the receptor is in the high affinity state due to the presence of saturating concentrations of G protein. These results provide increased understanding to how allosteric modulators influence the high affinity state and drive GPCR signalling.

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[2] Whorton M. R., et al. (2007) PNAS 104:7682-7687

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Calcium communication in an *in vitro* model of breast cancer brain metastases

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Introduction. The brain is unique among sites of breast cancer metastases for poor prognosis, cellular landscape, and limited treatment options. The interaction between breast cancer and brain cells is thought to contribute to metastatic survival and outgrowth, however, the understanding of these interactions is hindered in part by a scarcity of pre-clinical models. Human neural progenitor cells, such as ReNcell VM, can differentiate into matrices of astrocytes and neurons to provide a model of the brain microenvironment suited to high-throughput investigations of cellular pathways. Calcium signalling is deregulated in numerous pathologies, including cancers and their surrounding microenvironment. To date however, the role of calcium signalling in breast cancer brain metastases has been largely unexplored.

Aims. To investigate calcium signalling in breast cancer brain metastases in a high-throughput microenvironment model.

Methods. ReNcell VM stably expressing the calcium sensor jrCaMP1b were differentiated into matrices of neurons and astrocytes. MDA-MB-468 breast cancer cells stably expressing the calcium sensor GCaMP6m were added to neural matrices, and co-cultured for 7 days. Calcium signalling between cell populations was assessed through addition of 10 nM GSK1016790a to stimulate calcium influx selectively in breast cancer cells. Experiments were conducted with an ImageXpress^{MICRO} and single cell data was analysed via MetaXpress and MATLAB.

Results. Addition of GSK1016790a to co-cultures stimulated increased calcium influx in breast cancer cells and ReNcell VM, which was absent in ReNcell VM monoculture. Analysis of single cell signalling events in co-cultured ReNcell VM determined a relationship between increased calcium activity and proximity to breast cancer cells responding to GSK1016790a.

Discussion. This study provides the first evidence of calcium communication between breast cancer and the brain microenvironment, a potential avenue to explore for therapeutic targeting for this disease.

Joyce, J, Quail, D (2017) Cancer Cell 13;31(3):326-341
Monteith et al (2019) Cold Spring Harb Perspect Biol. 1;11(8):a035204

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Biased negative allosteric modulators for the calcium-sensing receptor have differential bronchodilator and bronchoprotective effects in mouse precision cut lung slices

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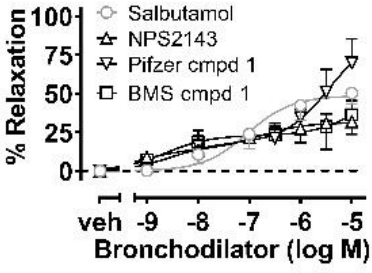
Introduction. The calcium-sensing receptor (CaSR) detects changes in extracellular calcium (Ca^{2+}_o) to maintain Ca^{2+}_o homeostasis. The CaSR is upregulated in asthma, and CaSR negative allosteric modulators (NAMs) reduce inflammation, remodelling, and airway hyperresponsiveness in a mouse model of chronic allergic airways disease (Yarova et al, 2015). Whether CaSR NAMs, which engender biased modulation (Davey et al, 2012), have different bronchodilator and/or bronchoprotective effects is unknown.

Aim. To assess CaSR NAM (NPS2143, Pfizer cmpd 1, BMS cmpd 1) bias in CaSR-HEK293 cells and compare NAMs with the β_2-adrenoceptor agonist salbutamol for airway relaxation.

Methods. Intracellular calcium (Ca^{2+}_i) mobilisation and IP_1 accumulation assays in CaSR-HEK293 cells were used to quantify the affinity and cooperativity of CaSR NAMs. Precision cut lung slices from male C57Bl/6 mice were prepared to visualise changes in airway area after contraction stimulated by 300 nM methacholine (MCh) followed by NAM or salbutamol (bronchodilation assays) or after overnight pre-incubation with 1 µM NAMs (bronchoprotection assays).



Results. CaSR NAMs engendered differential and biased modulation of Ca^{2+}_i mobilisation and IP_1 accumulation. CaSR NAMs relaxed pre-contracted airways in a biphasic manner (see figure), with the highest potency first phase of their response being 1000-fold higher potency than salbutamol. Salbutamol and NAMs caused comparable maximal bronchodilation (salbutamol 50±7%, NPS2143 32±8%, Pfizer cmpd 1 70±16%, BMS cmpd 1 48±16%, n=4-6). Overnight incubation with NPS2143 and Pfizer cmpd 1, but not BMS cmpd 1 prevented contraction.

Discussion. CaSR NAMs show differential effects on MCh-induced airway contraction, with Pfizer cmpd 1 exhibiting greater bronchodilator efficacy and potency than salbutamol. Confirmation of benefit compared to salbutamol in asthmatic airways would further support the CaSR as a novel therapeutic target for the treatment of asthma.

Yarova et al (2015) Sci Transl Med. 7:284
Davey et al (2012) Endocrinology. 153:1232

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The effect of single nucleotide polymorphisms on sweet taste receptor expression and cell surface trafficking

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Introduction. The sweet taste receptor (STR) is a family C GPCR consisting of two subunits, T1R2 and T1R3. It is expressed in oral and extra-oral tissues, including the pancreas and gut. The STR subunits are highly polymorphic and four T1R2 single nucleotide polymorphisms (SNPs) occur with ≥ 75% overall allele frequency, including T1R2 I191V and S9C which have been associated with decreased sugar consumption and lower waist-height ratios in obese individuals (Eny et al, 2010; Pioltine et al, 2018). Hence, the STR is a potential novel drug target for treating obesity and type 2 diabetes. However, the underlying mechanism by which these SNPs produce these phenotypes is not known.

Aims. To (i) build homology models of the T1R2 venus flytrap domain (VFD) to predict the effects of the SNPs on ligand binding and (ii) determine the effect of T1R2 I191V and S9C mutations on STR expression and cell surface trafficking.

Method. Using multiple VFD crystal structures as templates, homology models of the hT1R2 VFD were generated and docking of ligands was performed. T1R2 receptors containing either I191V or S9C were transiently transfected into AD293 cells, either alone or alongside wildtype T1R3. Biotinylation pull-down experiments and western blotting were performed to determine the effect of the SNPs on total STR expression and cell surface trafficking.

Results. Assessment of the VFD models indicated that the calcium sensing receptor and the medaka fish T1R3 crystal structures serve as the best templates for generating homology models of the human T1R2 VFD. The homology models predicted that both I191 and S9 are outside of the sucrose binding site. Preliminary results from surface biotinylation experiments suggest that neither T1R2 SNP significantly affected total STR expression or cell surface trafficking though T1R2/T1R3 co-expression appears to result in decreased T1R3 expression.

Discussion. The altered STR expression profile with co-expression of T1R2 and T1R3 may indicate that T1R2 suppresses T1R3 expression in the heterologous expression system used. As the T1R2 SNPs do not appear to alter STR expression or cell surface trafficking, the molecular mechanism underpinning the STR metabolic associations remains elusive.

Eny KM et al (2010) AM J Clin Nutr 92: 1501-1510
Pioltin MB et al (2018) J Pers Med 8:7-15

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Expanding the peptide synthesis toolkit to produce bicyclic peptide mimetics for drug discovery

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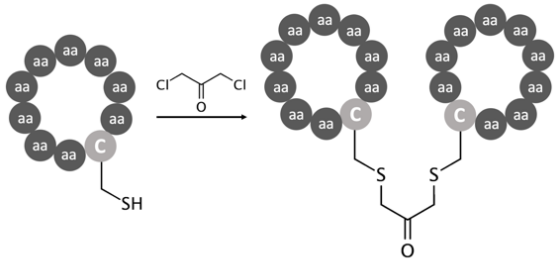
Introduction. The design and synthesis of cyclic peptides is a widely established practice in the field of peptide chemistry. This has been further expanded by the development of orthogonal chemical reactions allowing for production of more chemically complex peptides. We have recently reported a versatile method to produce bicyclic homodimer peptides that are selective mimetics of loops of large proteins, including neurotrophins.

Aims. Use 1,3-dichloroacetone (DCA) to selectively link free cysteine side-chains via an acetone bridge, producing bicyclic dimeric peptides.

Methods. Synthesised six backbone-cyclic peptides, each possessing a single cysteine residue, and created bicyclic dimeric peptides by linking two copies of the cyclic peptide together via an acetone linker using DCA. We systematically investigated a range of reaction conditions, including reaction stoichiometry, peptide concentration, pH and buffer composition.



Results. We were successfully able to identify the optimum conditions for peptide dimerisation for our six peptide sequences and have use these results to produce an overall guide for preparing acetone-linked bicyclic peptides. The peptides were subsequently analysed for proteolytic stability in human serum and were observed to still be fully intact after 48 hours.

Discussion. This study provides valuable insights into the use of DCA as a tool in peptide synthesis. The non-reducible nature of the acetone linker between pairs of cysteine residues makes the DCA dimerisation reaction attractive compared to the better-known disulfide bond approach.

Lin Q et al (2020) ACS Omega 5:1840-1850

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Characterisation of the G protein coupling profiles of PAC1 receptor splice isoforms

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Introduction. The pituitary adenylate cyclase-activating polypeptide (PACAP) type I receptor (PAC1R) is an attractive therapeutic target for the treatment of many CNS diseases including migraines and post-traumatic stress disorder (Rubio-Beltrán et al, 2018; Ressler et al, 2011). Extensive alternative splice isoforms of PAC1R have been identified. These isoforms contain alterations in the intracellular loop 3 (ICL3) and/or the N-terminal extracellular domain (ECD). Previous studies have suggested distinct signalling properties of these isoforms (Lutz et al, 2006). However, comprehensive characterisation of their transducer coupling, activation and regulation profiles is currently lacking.

Aim. In this study, we have characterised the G protein coupling profiles of PAC1R isoforms, including the most common splice isoform, termed PAC1 null (PAC1n) and variants with a truncated ECD (PAC1s), in addition to variants that contain ICL3 insertions (hip, hop or hiphop) using TRUPATH G protein biosensors.

Methods. PAC1R isoforms and TRUPATH biosensors were transiently transfected into COS-7 cells and treated with increasing concentrations of agonists: PACAP-38, PACAP-27, vasoactive intestinal peptide (VIP) and maxadilan. Real-time G protein dissociation profiles of G_{s}, G_{i}, $G_{q/11}$ and G_{12} were measured at 37°C using PHERAstar (BMG Biotech).

Results. Insertions in ICL3 altered the G-protein coupling profiles of PAC1n and PAC1s. PAC1n-hop displayed a four-fold increase in PACAP-38 potency for $G_{q/11}$ coupling (G_{q} pEC_{50}: 8.4±0.1; G_{11} pEC_{50}: 8.3±0.1) compared to PAC1n (G_{q} pEC_{50}: 7.8±0.2; G_{11} pEC_{50}: 7.8±0.2). While, insertions of the hip and hiphop cassettes led to weaker G_{i1} coupling. PAC1s increased the potency of VIP-mediated G protein coupling for all four G protein subtypes.

Discussion. Altered G protein coupling profiles of the PAC1R ICL3 variants contribute to their overall signalling profile, while splice isoforms in the N-terminal ECD reduced functional coupling to all G proteins and may be indicative of reduced ligand affinity. The results from this study provide insight into the signalling mechanisms of PAC1R.

Lutz EM et al (2006) Mol Cell Neurosci 31:193-209
Ressler KJ et al (2011) Nature 470:492-497
Rubio-Beltrán E et al (2018) J Headache Pain 19:64

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Understanding GPCR signal compartmentalisation using optogenetic methods

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Introduction. Eukaryotic cells possess a limited number of signaling proteins, yet can execute an extensive range of functional outcomes due to the spatiotemporal organisation of intracellular signalling pathways. Spatial compartmentalisation of G protein-coupled receptor (GPCR) signalling results in location-specific production of second messengers that can be recognised by the cell and translated into unique downstream responses. This dynamic concept can be investigated using novel optogenetic methods that offer targeted and highly specific activation of intracellular signalling pathways using light in lieu of ligands.

Aims. Demonstrate that targeting a GPCR to different subcellular locations changes the resultant cellular outcome.

Methods. Subcellular GPCR localisation was validated using confocal microscopy; cellular outcome was quantified using signalling assays and quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Results. The shared structural homology among GPCRs facilitates the modular conjugation of the ligand-sensing and transmembrane domains of one GPCR with the intracellular signaling domains of another. Using this approach, an optogenetic rhodopsin β2-adrenoceptor (opto-β2AR) chimera was created: a light-responsive GPCR that activates the canonical Gs-mediated signalling of the wild-type β2-adrenoceptor. At the plasma membrane, opto-β2AR mimics cyclic AMP (cAMP) production and internalisation comparable to the native β2-adrenoreceptor (Siuda et al, 2015). We targeted opto-β2AR to distinct subcellular locations (including early endosomes, golgi and mitochondria) where light activation stimulates cAMP production. Differences in transcriptional responses from spatially distinct pools of cAMP can provide insight as to whether receptor subcellular localisation controls unique cellular outcomes.

Discussion. Disease-relevant GPCR signaling can be location dependant: a greater understanding of signal compartmentalisation will challenge existing conceptions about plasma-membrane delimited signaling and encourage new strategies for GPCR-targeted drug discovery.

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

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Differential G protein activation kinetics may underpin the beneficial aspects of clinically trialled A1R atypical agonists

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Introduction. Activation of adenosine A_1 receptors (A_1Rs) represents a powerful strategy for the treatment of cardiovascular disease. Clinically trialled atypical A_1R agonists, capadenoson and neladenoson, stimulate cardioprotection (Sabah et al, 2013) with minimal bradycardia (Shah et al, 2019; Sabah et al, 2013), a signalling profile typically attributed to reduced intrinsic efficacy. However, capadenoson and neladenoson are biased A_1R agonists (Baltos et al, 2016; Rueda et al, 2020), and as such a better mechanistic understanding is required to facilitate the rational design of more effective A_1R therapeutic candidates. Aim. To quantify A_1R-mediated Gβγ-effector interactions in response to prototypical and atypical A_1R agonists; as Gβγ-GIRK channel interactions are the direct mechanism for A_1R-mediated bradycardia. Methods. Stably expressing A_1R-HEK293A cells with all G proteins deleted were transiently transfected with masGRKct-nanoluc, $G\alpha_{oA}$, and $G\beta_1\gamma_2$-venus. The masGRKct construct readily binds free Gβγ dimers, on a timescale that mirrors GIRK channel activation (Hollins et al, 2009). Results. The prototypical agonist MeCCPA and capadenoson were equipotent with a similar maximal response (pEC_{50}: 6.6 – 6.9; n=3-4, P>0.05). However, the onset kinetics of $G\beta_1\gamma_2$ interactions atypical agonists were significantly reduced by 4-8 fold as compared to prototypical agonists (n=3-4, P<0.05). Discussion. Considering the similar potency and maximal response observed for MeCCPA and capadenoson, the different kinetic profile of Gβγ-effector interactions may have a key role in conferring the clinically beneficial profile of atypical A_1R agonists.

Baltos J et al (2016) Biochem Pharmacol 99:101-112
Hollins B et al (2009) Cell Signal 21(6):1015-1021
Rueda P et al (2020) bioRxiv preprint doi: https://doi.org/10.1101/2020.07.22.215509
Sabah et al (2013) Circ Heart Fail 6:563-571
Shah et al (2019) JAMA 321:2102-2112

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GPCR-CoINPocket2.0: refining the prediction of liganded pharmacological neighbours of unliganded orphan GPCRs

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Introduction. We developed GPCR-CoINPocket (GPCR Contact-Informed Neighbouring Pocket), a crystal structure-informed metric predicting Family A GPCR pharmacological similarity[1]. In the range of 20-35% sequence similarity (too low for precise homology modelling but adequate for sequence alignment), GPCR-CoINPocket predictions outperform both transmembrane and binding pocket sequence similarity. Nevertheless, some known chemical relationships (e.g. between the CB_2 and Y_5 receptors) were not captured, indicating that improvements can still be made to the metric.

Aims. Here, we aim to improve the accuracy of GPCR-CoINPocket by increasing structure diversity, and developing a new residue comparison matrix (RCM) that more directly reflects residue similarity in ligand-binding characteristics.

Methods. We use large-scale GPCR crystal structure analysis to identify preferred receptor:ligand binding pocket contacts, ChEMBL database analysis to build chemical fragment clusters for RCM construction, and analysis of all structures in the Pocketome[2] to reveal preferences in interaction patterns between amino acid fragments and various chemical fragments.

Results. GPCR-CoINPocket now includes 254 structures (up from 116) from 38 GPCRs (up from 27). To reduce data granularity and increase signal, chemical compound fragments were clustered into groups based on their "interchangeability" within different scaffolds where fragment substitution had no or minor effects on the binding affinities to various targets. Backbone, polar, and non-polar distance-based contact strength distributions between fragment clusters and interacting residues were obtained by analysing all liganded structures released up until April, 2018. Work to generate and apply a RCM to GPCR-CoINPocket based on these distributions is ongoing.

Discussion. The development of a residue:ligand contact-based RCM for GPCR-CoINPocket should further improve the predictive accuracy of the metric. Identifying liganded pharmacological neighbours of unliganded orphan GPCRs guides the development of pharmacological tools with which the function and role of orphan GPCRs may be unlocked.

[1]Ngo T et al (2017) Nat Chem Biol 13(2): 235-242.
[2]Kufareva I et al (2012) Nucleic Acids Res 40: D535-540

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Evaluation of meropenem-tobramycin combination regimens against clinical hypermutable *Pseudomonas aeruginosa via* mechanism-based modelling and the dynamic hollow fibre infection model

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Introduction. Hypermutable *Pseudomonas aeruginosa* (HYPa) strains occur frequently in patients with cystic fibrosis and are associated with chronic respiratory infections leading to increased morbidity and mortality. These strains have increased mutation rates resulting in multidrug-resistance and treatment failure.

Aims. To characterise bacterial killing and emergence of resistance of clinically relevant meropenem (MER) and tobramycin (TOB) dosage regimens against two clinical hypermutable *Pseudomonas aeruginosa* isolates at simulated epithelial lining fluid (ELF) concentration-time profiles.

Methods. Hollow-fibre infection model (HFIM) experiments were conducted over 8 days for different dosage regimens of MER and TOB alone and in combinations against CW8 (MIC_{MER} = 8 mg/L, MIC_{TOB} = 8 mg/L) and CW44 (MIC_{MER} = 4 mg/L, MIC_{TOB} = 2 mg/L). The total and less susceptible bacterial populations were quantified and subsequently simultaneously described *via* mechanism-based modelling (MBM).

Results. Monotherapies and low dose combination regimens produced rapid regrowth. The highest daily doses for MER (2 g every 8 h, 3 h infusion) and TOB (10 mg/kg every 24 h, 30 min infusion) in combination demonstrated synergistic killing and resistance suppression below the monotherapy counts over at least 143 h for both isolates. MBM incorporating three bacterial subpopulations and direct bacterial killing by both antibiotics well described the antibacterial effects of the mono- and combination therapies in the HFIM.

Discussion. The MBM which were developed successfully accounted for the time course of amplification of pre-existing less-susceptible bacterial subpopulations. The MER-TOB synergistic combination is promising against clinical HYPa strains and warrants further evaluation.

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Synergistic ceftazidime and tobramycin combinations for clinical hypermutable Pseudomonas aeruginosa isolates; an innovative dosing approach to enhance bacterial killing and mitigate resistance in a dynamic biofilm model

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Introduction. *Pseudomonas aeruginosa* chronically infects patients with cystic fibrosis and is associated with increased morbidity and mortality. Ceftazidime and tobramycin are considered first-line treatments. However, hypermutability and biofilm formation results in treatment failure due to selection of resistant mutants.

Aims. We systematically investigated the pharmacodynamic effects of intravenous *versus* inhalation dosage regimens of tobramycin with and without intravenous ceftazidime.

Methods. Two clinical hypermutable *P. aeruginosa* isolates CW30 (MIC_{CAZ} 0.5 mg/L, MIC_{TOB} 2 mg/L) and CW8 (MIC_{CAZ} 2 mg/L, MIC_{TOB} 8 mg/L) were investigated for 120 h in the dynamic *in vitro* CDC biofilm reactor. Clinically relevant treatments were: continuous infusion ceftazidime 9 g/day (33% lung penetration); intravenous tobramycin 10mg/kg Q24h (50% lung penetration); and tobramycin 300 mg Q12h as inhalation, and their combinations. Total and less-susceptible planktonic and biofilm bacterial counts were carried out over 120 h. Ceftazidime and Tobramycin were quantified by LC-MS/MS.

Results. All treatments in monotherapy were ineffective for both isolates, with a regrowth of planktonic (≥4.7log_{10} CFU/mL) and biofilm (>6.6log_{10} CFU/cm^2) bacteria, and amplification of less-susceptible planktonic and biofilm bacteria by 120 h. Both combination treatments demonstrated synergistic bacterial killing, not only for planktonic but also biofilm bacteria; however, greatest bacterial killing against both modes of bacterial growth was observed with the combination simulating tobramycin inhalation. In addition, the combination regimens resulted in a very substantial suppression of resistance of planktonic and biofilm bacteria to each of the antibiotics for both isolates.

Discussion. Thus, ceftazidime combinations with intravenous or, especially, inhaled tobramycin hold promise to treat challenging infections caused by hypermutable *P. aeruginosa* strains and warrant clinical investigation.

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Effect of high Drug Burden Index polypharmacy on physical function and daily activities: male and female, young and old mice.

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Introduction. Polypharmacy (use of ≥ 5 drugs) and increasing DBI (DBI: measure of total exposure to anticholinergic and sedative drugs) are associated with impaired physical function, dependence in daily activities and increased frailty in observational studies of older adults. The effect of polypharmacy on sex is unclear.

Aims. To determine the effect of high DBI polypharmacy on these outcomes in male and female, young and old mice.

Methods. Young (Y, 2.5 months) and old (O, 21.5 months) male (M) and female (F) C57BL/6 mice received control (C) feed or high DBI polypharmacy (P) feed/water containing therapeutic doses of five drugs (simvastatin, metoprolol, oxybutynin, oxycodone, citalopram) (n=6-8/ group) for 4-6 weeks. Function was assessed before and after treatment.

Results. No statistically significant age or sex difference was observed for any outcome between age or sex groups within the control groups. Compared to control, polypharmacy decreased grip strength (GS) and rotarod latency (RL) for all age and sex groups (GS (N): 13.3 ± 6.8 YFC: -28.8 ± 9.4 YFP, 10.7 ± 3.6 YMC, -41.4 ± 16.0 YMP, 5.1 ± 2.6 OFC, -45. 0 ± 12.0 OFP, 5.5 ± 5.1 OMC, -42.8 ± 9.4 OMP; RL (sec): 28.3 ± 9.5 YFC, -13.8 ± 8.0 YFP, 28.6 ± 6.6 YMC, -42.5 ± 8.2 YMP, 17.8 ± 4.8 OFC, -16.8 ± 13.4 OFP, 24.2 ± 7.0 OMC, -10.2 ± 10.8 OMP, $p<0.05$). For RL, a greater decline was observed in young than old male animals following polypharmacy ($p<0.05$). Compared to control, polypharmacy reduced distanced travelled (m) in only male young and old animals (YFC: -3.0 ± 4.8, YFH: -13.6 ± 4.7, YMC: 0.8 ± 2.9, YMP: -9.7 ± 2.0, OFC: 0.5 ± 1.4, OFH: -7.0 ± 3.7, OMC: -2.4 ± 2.4, OMP: -12.3 ± 3.3, $p<0.05$).Compared to control, polypharmacy significantly reduced nesting ability in old female and male mice only (score: YFC: 0.2 ± 1.1, YFP: -1.7 ± 1.4, YMC: -1.7 ± 2.9, YMP: -5.8 ± 2.2, OFC: 1.3 ± 1.0, OFP: -3.6 ± 1.1, OMC: 1.4 ± 1.9, OMP: -5.9 ± 1.4, $p<0.05$).

Discussion. Our results show that high DBI polypharmacy impairs physical function in both sexes and ages, and some treatment effects may be specific to sex and age.

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The role of UGT enzymes as novel modulators of lipid biosynthesis and SREBP signalling in breast cancer

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Introduction. Elevated lipogenesis is a hallmark of cancer, often caused by an increase in the activity of the master regulators of lipid biosynthesis; sterol regulatory binding protein (SREBP) transcription factors. UDP-glycosyltransferases (UGTs) are a superfamily of enzymes that conjugate sugars to small lipophilic molecules including endobiotics, xenobiotics, and drugs. The expression of two UGTs that have poorly defined activities, UGT2B11 and UGT2B28, has been linked with pathogenic features of breast and prostate cancer. Analysis of the Cancer Genome Atlas Breast Cancer RNAseq dataset correlated expression of these UGT with genes involved in SREBP-mediated lipogenesis. Guided by this finding we investigated functional linkages of UGTs with SREBP signalling in cancer.

Aims. To define the roles of UGT2B11 and UGT2B28 in the regulation of SREBP-mediated lipogenesis.

Methods. UGT2B11 and UGT2B28 variants were stably expressed in MDA-MB-453 breast cancer cells. Cellular proliferation was assessed via crystal violet assay and SREBP lipogenic target gene expression was quantified by qPCR. UGT2B11 and UGT2B28 were transiently co-expressed with components of the SREBP signalling complex in a HEK-293T cell model. The stability of nuclear SREBP protein was assessed via immunoblotting and changes in SREBP transactivation function was quantified using luciferase reporter assays.

Results. Stable overexpression of UGT2B11 and UGT2B28, including active full-length forms and catalytically inactive truncated variants, promoted breast cancer cell proliferation. Gene expression analysis revealed increased levels of multiple SREBP target genes in the UGT-overexpressing cells. Co-expression studies in HEK-293T cells showed that these UGTs can enhance proteolytic turnover of nuclear nSREBPs, leading to reduced transactivation activity.

Discussion. Expression of UGTs appears to enhance SREBP-mediated lipogenesis and proliferation in breast cancer cells. This may involve modulation of the ER-based lipid sensing process that controls nuclear trafficking of SREBP, likely via a non-catalytic mechanism as truncated and full length UGTs had similar effects. The ability of these UGTs to modulate proteolytic turnover of nuclear nSREBPs could terminate transactivation function. Taking these findings together we propose a mechanism whereby UGTs control the balance between activation and termination of SREBP signalling. The finding that UGTs may be novel regulators of lipid biosynthesis may help explain their association with poor breast cancer outcomes and prompts their further investigation as novel biomarkers or therapeutic targets.

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Survey of how and why students use resources in a pharmacology course

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Introduction. Students can often select between a variety of resources e.g. attending face-to-face lectures, accessing lecture recordings, and standalone Powerpoints.

Aims. To survey students to determine how and why students use these resources in a pharmacology course.

Methods. Online survey, with students studying pharmacology to tick all that apply.

Results. The study was undertaken in 2017 and 2019. Only 17% of students consented to undertake the study. About half of the students attended lectures. The three most common reasons for attending lectures were '*It allows for interaction with unit staff and/or students'*, *'I am concerned that recordings may not be complete or the technology for recording may fail'* and *'I think I learn more by attending'*. For those students who accessed lecture recordings as well as attending lectures, the two most common responses were '*Revise lecture concepts for assessment purposes'* and '*clarify difficult concepts'*. For those students who did not attend lectures, reasons for not attending; '*I don't like the lecture theatre environment'* and '*I don't like the lecture time – it was too early/late'*. Those that did not attend lectures but accessed lecture recordings; '*I prefer the flexibility of the online recordings'* and '*I don't like the lecture theatre environment'.* The final question on the survey was to ask the students to add any additional comments or feedback they had on the use of lecture recordings as a learning tool, and the main themes were '*flexibility*' and '*useful*'. A high percentage of students used standalone Powerpoints as a resource, mainly to study prior to assessment or exams.

Discussion. Despite consent being sought in workshops and lectures, which were both poorly attended, and online, the number of participating students was low. However, as the consenting students had similar marks to the overall class marks, the participants may have been representative of the class. In conclusion, it seems appropriate to supply students with a range of resources, as individual students use them and appreciate them for a range of reasons.

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An examination of extemporaneous compounding skills, knowledge and confidence progression from undergraduate to practice: a pharmacy education perspective

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Introduction: Extemporaneous compounding is a required component of the pharmacist registration. A competent pharmacist is expected to have appropriate education, training and skills to undertake extemporaneous compounding of safe and efficacious products. While there have been some studies on compounding practice of pharmacists, little is known about the educational aspect of extemporaneous compounding such as how pharmacy students perceive their training and progression from undergraduate to internship.

Aim: Our study aimed to examine how pharmacy students, interns (pre-registrant pharmacy graduate) and pharmacists perceived their competency in extemporaneous compounding of pharmaceutical products.

Methods: We designed and conducted a self-administered survey with undergraduate pharmacy students, interns and pharmacists. Using a Likert-scale, participants ranked how confident they felt in compounding a selection of extemporaneous products. The data were then analysed with Kruskal-Wallis test. Participants' opinions on areas of improvement of extemporaneous compounding teaching were also recorded as free-text responses.

Results: 27 undergraduate students, 7 interns and 7 pharmacists completed the questionnaire. Compared to students, pharmacists perceived themselves to be significantly more confident in compounding suppositories and pessaries ($p = 0.013$), while showing no statistical differences in other products such as solutions, suspensions, creams and ointments. There was no significant difference between perceptions of undergraduate students and interns on their competence to compound "simple" pharmacy products. Various factors contributed to participants' perceptions including level of knowledge, training and experience. Suggestions to improve teaching extemporaneous compounding curriculum included frequent laboratory-based practice, integration of theoretical knowledge, legislation, fostering soft skills and clinical aspects.

Discussion: Pharmacy students participated in our study perceived they could competently perform simple compounding of pharmaceuticals. Nonetheless, extemporaneous compounding curriculum can further be improved enhanced to optimize student knowledge, skills and learning experiences in this unique area of professional practice.

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Communicating health risks of nicotine vaping products: a systematic review of message content, format, and source on harm perception and behavioural intentions

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Introduction. Effective ways of communicating relative risks of various nicotine-containing products can help increase the accuracy of relative harm perceptions of nicotine vaping products (NVPs) compared with combustible cigarettes and increase smokers' intentions to quit smoking and/or to switch to vaping.

Aims. To systematically review the literature on (1) whether and how various risk messages about NVPs alter harm perception and behavioural intentions of smokers and non-smokers, and (2) how trust in sources of NVP risk communication affects message reception and behavioural intentions.

Methods. Seven electronic databases (PubMed, PsycINFO, EMBASE, Web of Science, Communication & Mass Media Complete, CINAHL, and Google scholar) and reference lists of relevant articles were searched for articles published up to April 2020. Experimental, quasi-experimental, and cross-sectional studies were included. The Newcastle–Ottawa Scale and the Evidence Project Risk of Bias Tool were employed to assess the quality of observational and intervention studies, respectively. Key findings were extracted and grouped into subcategories according to the Message Impact Framework.

Results. Nicotine addiction messages resulted in greater health and addiction risk perceptions, relative risk messages comparing the health risks of NVPs to cigarette smoking increased the perception that NVPs are less harmful than combustible cigarettes, and a nicotine fact sheet corrected misperceptions of nicotine and NVPs. Experimental studies found that smokers' intention to purchase, try or switch to NVPs was higher when exposed to a relative risk message and lower when exposed to nicotine addiction warnings. Trust in NVP risk information from public health agencies was associated with lower odds of: i) NVP use, and ii) perceiving NVPs as less harmful, whereas those who trusted information from NVP companies were more likely to perceive NVPs as less harmful than combustible cigarettes.

Discussion. Our findings suggest that relative risk messages can help improve the accuracy of harm perceptions of NVPs and increase smokers' intentions to quit smoking and/or to switch to vaping, although the literature is nascent. Future research should explore the most effective way of pairing relative risk messages with warning labels (such as nicotine addiction) in order to maximize potential public health benefits while minimizing unintended consequences.

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Integrating virtual simulation (MyDispense) for teaching comprehensive care pharmacy curriculum.

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Introduction. The integration and application of knowledge to solve medication-related problems is a critical skill for pharmacists. This skill is commonly taught through case-based teaching. However, this technique does not require students to gather and summarise all relevant patient-specific information on their own. MyDispense, a web-based pharmacy simulation program, allows students to assume the role of a pharmacist to evaluate, verify, and dispense a prescription in a virtual pharmacy setting.

Aims. To utilise new and existing case-based teaching material to create MyDispense simulation activities to teach 2nd year comprehensive care (CC) Pharmacy curriculum.

Methods. In the latter half of 2nd year, Pharmacy students undertake a unit focused on knowledge and skills required for the diagnosis and therapeutic management of patients with various endocrine and renal conditions. New and existing case materials were adapted to create two standard MyDispense teaching activities that were integrated into the endocrine and renal CC curriculum. A third MyDispense activity was created and integrated into a cardiovascular focused CC unit which focussed on the integration of knowledge across CC units (thyroid, diabetes and hypertension).

Results. During the adaptation phase, two Clinical Practitioners reviewed existing case-based teaching material and incorporated new elements to the cases, focusing on appropriateness of therapy based virtual history taking, thereby simulating the identification, resolution and documentation of medication related problems. A secondary review was conducted and cases were built onto the MyDispense web-based platform by an experienced pharmacist. Cases were designed with a primary focus of teaching contraception and diabetes patient-centred management and counselling. The complex case produced allows students to apply their knowledge of management of a complex range of ailments. In addition, the design of the complex case included real-life complexities such as prioritisation of tasks, conflict resolution and necessity of patient centred care to better simulate an authentic experience.

Discussion. Virtual simulations such as MyDispense, offer an authentic teaching tool for Pharmacy curriculum. The standardised experience will allow the authors to evaluate students' perceptions on the effectiveness of using a virtual simulation (Mydispense) to improve patient-centred care.

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Implementing and Evaluating a Course in Professional Ethics for the Undergraduate Pharmacy Curriculum in Jordan

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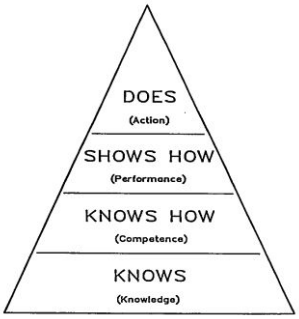

Miller's Model of Competence- Milers Pyramid.

Introduction. Today, pharmacy practice mandates "patient centred care" at a fundamental level. This patient centeredness assigns a higher level of responsibility for pharmacists. It is expected pharmacists are able to handle challenges competently and in the best interests of the patient. These challenges often involve ethical principles, institutional, personal or other constraints that can pull practitioners in incompatible opposite directions, leading "ethical dilemmas" in many circumstances. Literature underlines the positive impact of educational interventions focussing on ethical awareness and competence, and that 'gaps' were found to exist in pharmacy training/curricula for Jordanian pharmacists.

Aims. To develop, implement and evaluate the utility of a carefully designed ethics education component in pharmacy undergraduate curriculum for a cohort of pharmacy students within a well-ranked Jordanian University.

Methods. Fifth- year pharmacy students at ASU Applied Science Private University in Jordan, attending Summer Pharmacy School from July- September 2020 were invited to participate in an educational intervention (the delivery of a suite of didactic lectures online, followed by skills-based workshops online, to enable translation of theory into practice). This study was delivered in three main phases, with a pre-test and post-test survey administered immediately before and after the educational intervention. And focus-group discussions at the end to elicit students' feedback.

Results. Preliminary data indicated enhanced levels of confidence in students' decision-making. Moreover, the development of students' moral reasoning and decision-making skills were also observed to be enhanced.

Discussion. This study highlighted the importance of the implementation of an ethics course in pharmacy undergraduate curricula. This course made a positive impact on the students learning experience and provided a strong environment for discussion and group learning.

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Development and delivery of a pharmacology fundamental unit for first-year undergraduate students

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Introduction. In 2016, the Faculty of Medicine and Health Sciences released the Bachelor of Clinical Science, an innovative and accelerated degree with the aim to build foundational skills needed for a career in health. Pharmacology was not initially taught in this degree but during the first review in late 2017, I have strongly recommended the addition of a fundamental pharmacology unit due to clear benefit it would bring to future health professionals. The review process led to a curriculum redesign which included a pharmacology fundamentals unit to be implemented in 2019. The challenge became to teach pharmacology in an engaging manner to a cohort of first-year students without a good understanding of molecular biology and physiology. This was further aggravated by COVID-19 in 2020, which required changes to deliver the unit fully online as well as mixed mode.

Aims. To describe the process and considerations during development and delivery of a pharmacology fundamental unit to first-year Bachelor of Clinical Science students.

Methods. I have combined my teaching philosophy “Too much information is no information thus it is about quality, not quantity; and focus on keeping it relevant", and the information provided in the literature and by colleagues to develop this unit. I kept the material to the minimum focusing on important pharmacological concepts and integrated several games and clinical scenarios to engage the students. I also developed and implemented a pharmacodynamics online module for revision (student-centred approach). Short summative quizzes were developed to provide continuous feedback to the teaching staff on what is not understood specially considering the lack of basic knowledge in other related subjects. To keep it relevant to their future desired careers, one of the assessments is a group role-play where students must interpret drug profiles and apply knowledge acquired. Last, with the changes under COVID-19 restrictions, activities were modified to be available fully online (via zoom and teaching platform) to guarantee equity as some students are enrolled fully online because of health concerns around attending face-to-face tutorials.

Results and Discussion. Formal and informal feedback from students show that the unit has been well received. Students praised game-based approach and activities where clinical scenarios were used. In the current delivery, students appreciate the effort to deliver similar material to both face-to-face and fully online cohort. Formal feedback obtained this year will be used to better evaluate the online delivery and further improve this unit in 2021.

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Practising teamwork begins in the classroom: tools to evaluate teamwork behaviour in small group activities

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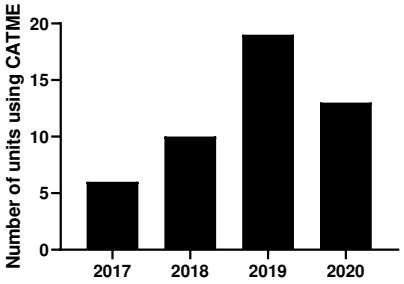
Introduction. Teamwork is a highly sought ‘soft-skill’ of graduates entering the workforce. In the classroom, students may work in groups but often forgo the opportunity to practise and develop teamwork behaviours to enable shared goals and shared learning.

Aim. To use a targeted approach to support the development of teamwork skills in undergraduate students using Comprehensive Assessment of Team-Member Effectiveness (CATME) and FeedbackFruits teamwork evaluation tools.



Methods. Numerous units at the Faculty of Pharmacy and Pharmaceutical Science at Monash University involve students working in semester-long predefined teams during small group teaching activities. Initially, students were taught effective teamwork, establishing team expectations and setting ground rules in the form of a team contract. Web-based team member evaluation tools CATME and FeedbackFruits were then embedded into units featuring small group work to provide information, demonstration, practice and feedback to students on various teamwork behaviour attributes.

Results. Since 2017, when the Faculty opted for a uniform approach to the evaluation of team member performance in team tasks, CATME has been utilised in 23 individual units across the two undergraduate degrees offered at the faculty. Subsequently, CATME was embedded within units to allow students the opportunity to evaluate, moderate and remediate their teamwork behaviour where needed. Positive reinforcement from students on the value of CATME for individuals working in teams and ability for instructors to monitor team dynamics and moderate individual grades for team tasks has seen the use of CATME grow over the last four years (see Figure 1). This year FeedbackFruits was piloted in 5 units across the faculty as an alternative tool for teamwork evaluation.

Discussion. Both CATME and FeedbackFruits offer useful tools to support teamwork behaviour development in students. FeedbackFruits provides a suite of additional tools such as peer review, interactive document and interactive presentation, making this web-based platform attractive for use in undergraduate curriculum.

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Prevalence and predictors of health information overload in patients with a chronic condition: A national survey

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Introduction. With the rapid growth of health information, patients with a chronic condition (e.g. diabetes) are at risk of developing health information overload (HIO). Generally, HIO can be defined as the point where the volume of the information exceeds the information processing ability of a particular individual. Feeling overloaded can impact processing and decision-making when dealing with health information.

Aims. This study aims to quantitatively determine the extent of HIO in Australian patients with diabetes, and to identify predictors of HIO in these patients.

Methods. Participants were recruited to take part in an online Australia-wide survey through Facebook from Dec 17th 2019 to Jan 2nd 2020. Inclusion criteria: adults aged 18 years and above, living in Australia, diagnosed with diabetes, and searching for, receiving or utilising health information regarding their diabetes in the English language. The Cancer Information Overload Scale was adopted (5 items using 5 point-Likert scales; total score from 5 to 25) to measure levels of HIO. A multiple linear regression model was used to identify the study variables that were associated with HIO.

Results. Of 455 participants, 73.2% were female and 60.8% were married or in a domestic partnership. The mean age of participants was 59.2±11.1 years. Preliminary data analysis showed that participants had a mean score of 14.2±4.33 for HIO. The following variables were significantly associated with HIO: type of DM (0.157, CI: 0.526-2.271), treatment burden (0.308, CI: 0.111-0.218), health literacy (-0.190, CI: -2.723- -0.783), and e-health literacy (-0.129, CI: -0.133- -0.012). Discussion. Our findings will help in providing deep insights into the prevalence, predictors and effects of HIO in patients with diabetes. The research outcomes will help healthcare professionals in terms of providing suitable amount of information that can be effectively used by patients with chronic health conditions.

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Students in the driving seat – online delivery of real time interactive practical classes

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Introduction. The undergraduate level 3 subject, Drugs in Biomedical Experiments, is a practical-based subject that exposes students to the experimental basis of scientific enquiry and enables them to develop skills relevant to contemporary biomedical research. The COVID-19 pandemic and health measures to limit community transmission have presented considerable challenges for delivering practical class teaching when students are unable to be physically present in the laboratory to conduct experiments.

Aim. Our aim was to redesign for online delivery, practical classes that challenge and engage students in enquiry-based learning and fulfill subject learning objectives.

Methods. Pre COVID-19, students worked collaboratively in groups to formulate hypotheses that could be investigated using isolated tissues in tissue organ bath systems. We adopted a novel approach of using proxies in the practical class to physically conduct experiments under the direction of groups of students who attend live via the video conferencing platform, Zoom. Chart recordings of tissue responses were live-streamed to students enabling them to instantaneously observe and interpret generated data. Data files were provided to students for data analysis via the Learning Management System.

Discussion. Use of proxies enabled student-designed experiments to be conducted in real-time during practical sessions. Anecdotal evidence suggests that student engagement is enhanced by the 'real' nature of the experiments and the ability to visualise in real-time, pharmacological phenomena of their own design. Live streaming of the chart recorder and upload of data files provided the flexibility for student groups to adjust or extend their experimental protocols accordingly. Key enabling factors were (a) maintaining the same online groups throughout the course to enable students to build rapport and develop interpersonal collaborative learning skills (key graduate attributes), and (b) providing several lead in online workshops, video and CAL based lessons to equip students with sufficient background knowledge of quantitative pharmacological analysis and relevant tissue systems to develop their own hypotheses and experimental plan. Online practicals are prone to student disengagement. Here, we adopted an innovative approach to deliver real time interactive practical classes online that were successful in engaging students and fulfilling the desired learning outcomes.

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Using PollEverywhere and content chunking during remote delivery in an undergraduate course

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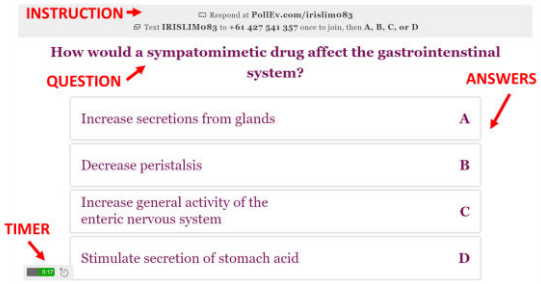
Introduction. In line with the COVID-19 lockdown and social distancing policies, most educational institutions have suspended face-to-face learning activities. Academics are challenged to embrace digital pedagogy while creating and improving effective student engagement strategies to ensure learning enhancement in an online space.



Aims. The aim was to optimise the usage of the PollEverywhere online app as a tool to improve content chunking in remote lecture sessions.

Methods. Lecture notes were uploaded to the Learning Management System (Blackboard Ultra) prior to the lectures for optional reading. Topic introductory videos (5-7 minutes) were also made available for mandatory viewing. The weekly 2-hour lecture sessions were delivered synchronously via Blackboard Collaborate. The content was carefully divided into 20 – 25 minute sections, followed by 3 to 4 Multiple Choice and True/False Questions displayed using the PollEverywhere plug-in within Microsoft PowerPoint (see figure). A timer of one minute was set for each question to allow students to enter their answer using the PollEverywhere app on their smartphone or via the website link. Correct answers were then displayed and further discussed by the educator. Students were encouraged to register under the PollEverywhere system for identification, as their number of correct answers were tallied at the end of every session.

Results. Both quantitative and qualitative feedback from students indicated that this teaching approach was highly valued. Retention of 'live' participants in the remotely delivered lecture sessions were maintained throughout the semester, similar to that of face-to-face lectures in previous semesters. To encourage participation in this activity, a 'Top 10 PollEverywhere Leaderboard' was updated weekly in the course site and at the end of the semester, the three students with the highest scores received prizes in the form of online gift cards.

Discussion. Using polling and content chunking appears to be an effective way to maintain student engagement in a digital space and retaining student attendance and participation in synchronous teaching sessions.

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iiBalls: an updated and expanded iris simulation to teach autonomic pharmacology and diagnostic ocular drugs

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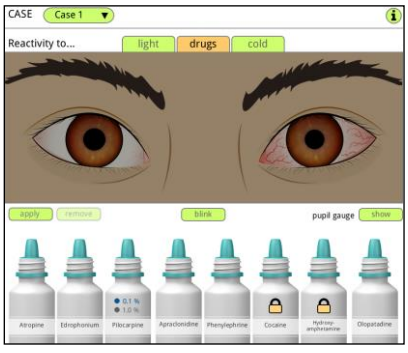

Introduction. The smooth muscle of the iris, with resultant change in pupil diameter, provides a clear means of illustrating the activity of the autonomic nervous system and its associated pharmacology. In addition, differential reactivity to a range of pharmacological agents is an important part of the diagnosis of many ocular conditions. We have previously generated an iris simulator (iBalls) and have successfully used this in teaching both Science and Optometry students. This simulator was further refined and then marketed by *Sheffield Bioscience Programs*. Here, we have updated and expanded iBalls to incorporate drug choices that are better aligned with optometry practice and expand the clinical cases to reflect the broad utility of drugs in an ocular diagnostic setting.

Aim. To generate and evaluate an engaging and clinically accurate computer simulation of the iris to better teach autonomic pharmacology and ocular diagnostics.

Methods. The iris simulator, choice of clinical cases and drug selection were developed and implemented by an interdisciplinary team. Given the strong relevance to optometry practice, the simulator was initially evaluated by year 2, Doctor of Optometry students, as a component of subject "Pharmacology for Health Professionals". Given its introduction in Semester 1, 2020, the class was run as a synchronous Zoom session. A brief anonymous questionnaire was used as the evaluation instrument. In this first iteration, no comparisons with the prior or alternative simulators were conducted.

Results. Student feedback was positive, confirming that the cases (*e.g.* Horner's syndrome, myasthenia gravis, Adie's tonic pupil) were highly engaging and relevant, and enabled them to better understand and apply the autonomic pharmacology lecture content they had received.

Discussion. An updated iris computer simulation (iiBalls) was generated and trialled with initial positive feedback. Future work aims to more comprehensively establish the learning efficacy of this new simulator not only for optometry students, but also for other student cohorts where understanding autonomic pharmacology is important.

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Exploring Transitional Challenges in Preparing Pharmacy Students for Practice

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Introduction: Pharmacy, at its core, is an amalgamation of many specialties, the subjects of which are often separate degrees. From an education perspective, pharmacy is uniquely problematic to define; it is difficult to draw a line between what is necessary to create 'medicines experts', and what is superfluous in preparing students for practice.

Aim: We aim to discover the core challenges faced by pharmacy students and pharmacists in their transitions to university and clinical practice, allowing future enhancements within pharmacy education to better prepare pharmacy students for practice.

Method: Our study employed a qualitative descriptive study design using interviews to gather information from student, pre-registration, and registered pharmacists concerning their education and practice experiences.

Results: The participants attributed the main challenges of their transition to university (n=46) and registered practice (n=18) involved being in a new environment, familiarity and application of knowledge, availability of support, and time management. Many participants identified having additional placements would supplement their pharmacy education and strengthen both clinical and non-clinical skills.

Discussion: It appears that students need more preparation and assistance to ensure smoother and more successful transitions in their careers. It is important for pharmacy degrees to change to reflect pharmacists' evolving scope of practice. Enhancing the development of high quality pharmacy graduates will result in pharmacist's who are prepared to embrace the diversity of pharmacy practice.

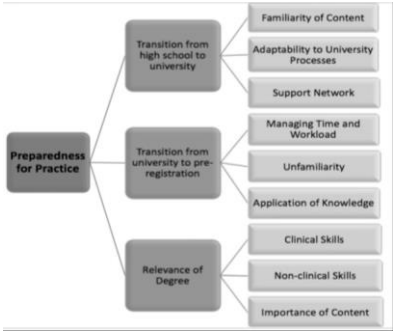

Figure 1: Preparedness for Practice Results

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Improving communication with patients with regards to the risk of SGLT2 inhibitor-associated diabetic ketoacidosis

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Introduction. It is important that patients are informed and aware of situations to withhold their sodium-glucose cotransporter 2 (SGLT2) inhibitor (acute illness and pre-surgery) to reduce the risk of diabetic ketoacidosis (DKA).

Aims. To explore the safety advice that general practitioners (GPs) and endocrinologists provide to patients taking SGLT2 inhibitors, and to explore patients' understanding of these safety issues.

Methods. Semi-structured interviews were conducted with 14 GPs, 11 endocrinologists and 13 patients taking SGLT2 inhibitors for type 2 diabetes. A snowballing approach was used to recruit GPs and endocrinologists. Emergent themes were identified from the transcripts.

Results. There are potential barriers to patients being aware of situations to withhold their SGLT2 inhibitor, particularly the situation of acute illness with reduced intake. These barriers include variable awareness and knowledge of the risk of DKA at a primary healthcare level. Additionally, if patients are informed of the risk of DKA and situations to withhold their agent, this information is often only provided at the time of prescription and in verbal form. Patients are generally not questioned in subsequent consultations about their understanding of the information initially provided. Therefore, potential knowledge deficits are not addressed. Furthermore, Consumer Medicine Information leaflets are generally not perceived by patients to be an effective means of communicating safety issues because of the volume, technicality and font size of the material.

Discussion. To improve patients' awareness of ways to reduce the risk of SGLT2 inhibitor-associated DKA, their needs to be ongoing education of GPs by endocrinologists about this serious adverse effect including risk factors for its occurrence. We recommend that following the prescription of an SGLT2 inhibitor clinicians question their patients about their understanding of situations to withhold their agent as this may identify that further reinforcement regarding this issue is required.

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COVID-19 and emergency online delivery: What worked for a pharmacology course?

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Introduction. The COVID-19 represents unique challenges to the education sector across the globe. Due to restrictions advised by the Australian health and foreign affairs authorities, including suspension of all in person class-room activities, Australian universities have transitioned to remote teaching and learning in Semester 1 2020.

Aims. The aim of this study is to describe the emergency move to online delivery of a pharmacology course and highlight the teaching approaches implemented during the COVID-19 pandemic to maintain education of students.

Methods. Within the basic structure of social distancing, compulsory online lecture delivery, and cancelling or postponing of all in class-room tutorials and practical classes, as course co-ordinators, we defined the scope of delivery issue and feasibility and proposed effective solutions for continuing delivery of lectures, tutorials, and practicals remotely.

Results and Discussion. A total of 58 DVM (Doctor of Veterinary Medicine) students were enrolled in the Pharmacology course in 2020. Before restrictions, the course was taught via three main in-person activities until week 4 of Semester 1, which include lectures, tutorials and practical classes comprised of clinical case studies and drug calculations. From week 5, the 23rd March 2020, the course was moved to on-line completely. Lecture recordings, produced by Echo360 universal, were posted online for students before the scheduled lectures time, at each of the teaching weeks via MyUni. Timed-online quizzes (1 hour duration/each, in MCQs & SAQs format) were administered via MyUni to replace face to face tutorials, supplemented with "summaries of key points" from lectures and "tutorial practice questions", both in pdf files made available online for students to access via MyUni. Online practicals (3 hour duration/each) were administered via MyUni using discussion boards, with the use of google docs to facilitate group discussion and report writing. Additional zoom meetings with students were also provided to clarify lecture, tutorial contents if needed, also allowing students to ask questions. All online class activities were maintained as per the regular weekly timetable as scheduled at the beginning of the semester, with no change to timing of lectures, tutorials or practicals.

Conclusion: In summary, the pharmacology course was successfully delivered in Semester 1, with all teaching activities completed timely. All 58 DVM students sit the primary online final exam and successfully completed the course.

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Type 2 diabetes – A risk of underdiagnosis in those with risk factors for diabetes

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Introduction: Glucose dysregulation refers to a group of conditions on a continuum that starts with early impaired fasting glucose (IFG) with further dysregulation preceding diagnosis of Type 2 diabetes (T2D)[1]. A person may be asymptomatic for many years before diagnosis and have some of the complications, including cardiovascular disease, retinopathy and neuropathic pain before diagnosis. For the general practitioner, the lack of recognisable risk factors, and the potential for standard diagnostic tools to provide an incorrect diagnosis, may lead to a small number of people being incorrectly diagnosed and fail to have appropriate measures in place to prevent complications[1, 2].

Methods: To assess reliability of fasting blood glucose (FBG) (assessing liver function in the fasting state) and post-prandial blood glucose (PBG) (assessing the fed state glucose metabolism dynamics) in people with a risk factor of T2D.

Results: The preliminary results from 242 separate glucose tolerance tests has indicated 10% discrepancy between the results of the FBG tests and the PBG tests.

Discussion: These results indicate that FBG levels, when used a single diagnostic marker without taking into account other risk factors, may be underestimating the prevalence of pre-diabetes or T2D by up to 10% in Australians and that further investigations should be performed for those with risk factors for T2D.

1. American Diabetes Association (2019) 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2019. Diabetes Care 42(Supplement 1): S13-S28
2. Chilelli NC et al (2014) Screening with HbA1c identifies only one in two individuals with diagnosis of prediabetes at oral glucose tolerance test: findings in a real-world Caucasian population. Acta Diabetologica 51(5): 875-82

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Meat in the sandwich? – Effect of the pharmacy residency program on pharmacy educators

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Introduction. A residency training program for early career hospital pharmacists was introduced by the Society of Hospital Pharmacists of Australia (SHPA) in Australia in late 2016, modelled on similar programs in other countries. Hospital pharmacy educators have the role of implementing the program's vision, reconciling workplace and pharmacy resident needs and demands.

Aims. This qualitative study explored pharmacy educators' early experiences in implementing the SHPA pharmacy residency program and their navigation of potentially conflicting needs of workplaces and residents.

Methods. Two focus groups and two semi-structured interviews were conducted with educators from ten residency sites. Audio recordings were transcribed verbatim and analysed using thematic analysis.

Results. Fourteen pharmacy educators and clinical pharmacists involved in implementing and delivering the pharmacy residency program participated in this study. Educators involved in the SHPA residency program identified that it provides a framework to structure the workplace based development of early career pharmacists. The implementation of the program placed significant demand on resources in workplaces which led to unexpected trade-offs, with residents having priority access to certain training opportunities. This led to concerns about the inadvertent development of a two-tiered system, in which educators have to reconcile limited resources and equitable access to developmental opportunities for resident and non-resident pharmacists.

Discussion. The SHPA pharmacy residency provides a structure for workplaces to implement consistent workplace based training for early career hospital pharmacists. Due to extra demand on resources and hospital pharmacy educators, who are generally the implementers of the program, potential risks of preferencing pharmacy resident training and opportunities over others need to be monitored and mitigated for.

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The FFA4 agonist TUG891 relaxes mouse airways by inhibiting calcium oscillations but not sensitivity in precision cut lung slices.

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Introduction. Excessive contraction of airway smooth muscle is a key feature of asthma pathophysiology, mediated by the parallel signaling pathways of calcium oscillations (due to release and reuptake by the sarcoplasmic reticulum) and calcium sensitivity. The β_2-adrenoceptor agonist salbutamol (SALB) inhibits both pathways, resulting in bronchodilation. However, its efficacy is reduced with increasing disease severity, prompting the need to identify novel dilators. The G-protein coupled receptor free fatty acid 4 (FFA4), expressed in airway smooth muscle, is activated by the synthetic agonist TUG891. The aim of this study was to compare bronchodilator responses to TUG891 and SALB and define the effects on of TUG891 on methacholine (MCh)-induced calcium oscillations and sensitivity.

Methods. Male 6-8 week BALB/c mice were euthanised, their lungs inflated with agarose and sliced with a vibratome to make precision cut lung slices (PCLS, 200-250µm thickness). Airway responses were measured as changes in airway area using phase contrast microscopy. Concentration-response curves to TUG891 and salbutamol were prepared in perfused PCLS after airways pre-contracted with MCh. To assess the effects of TUG891 on calcium sensitivity alone, some PCLS were treated with caffeine/ryanodine (caff/ry) to abolish MCh-induced calcium oscillations. Separate PCLS were loaded with SBP and Oregon green dye and fluorescence imaging was used to capture the effects of TUG891 on the increase in frequency of calcium oscillations induced by MCh.

Results. TUG891 was ~25% more efficacious than salbutamol in mice PCLS, with a lower potency (5.8±0.2, n=5) than salbutamol (4.9±0.3, n=4) ($p<0.05$). The highest concentration of TUG891 (100 µM) caused ~80% relaxation. After caff/ry treatment, pre-contraction to MCh was maintained but slower, relaxation to salbutamol was reduced while the dilator response to TUG891 was abolished. TUG891 completely prevented MCh-induced calcium oscillations at >1µM.

Discussion. TUG891 has higher efficacy than salbutamol, with bronchodilation mediated via potent inhibition of calcium oscillations, but no effect on calcium sensitivity. The different receptor targets and mechanisms of airway relaxation of TUG891 and salbutamol suggest that TUG891 may be an alternative or adjunct therapy when β_2-mediated relaxation is compromised in severe asthma.

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Ebselen prevents cigarette smoke-induced endothelial dysfunction in viral-induced exacerbations of COPD.

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Introduction. Chronic obstructive pulmonary disease (COPD) is characterised by severe airflow limitation, lung inflammation and significant oxidative stress, largely caused by cigarette smoke (CS) exposure. Globally, COPD is the 4th leading cause of death, with 50% of these patients dying from a cardiovascular event. Annually, patients experience up to 3 acute exacerbations of COPD (AECOPD) from viral and/or bacterial infection, which further increases the risk of mortality by 30%. Systemic inflammation and oxidative stress promote systemic vascular remodeling and atherosclerosis, however, the underlying mechanisms driving cardiovascular comorbidities in AECOPD remain unknown.

Aim. To define the mechanism driving cardiovascular comorbidities in AECOPD and examine the effectiveness of an antioxidant compound ebselen.

Methods. Male BALB/c mice were exposed to either room air (sham) or CS (9 cigarettes/ day, 5 days/week) for 8 weeks followed by intranasal inoculation with influenza A virus (Mem71, 1 x $10^{4.5}$ pfu) and culled 3 and 10-days post-infection. Mice were orally gavaged once daily with ebselen (10mg/kg) or vehicle (5% CM-cellulose) 1 h prior to the initial CS exposure of the day and during the influenza infection period. 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 investigate endothelial and smooth muscle dilator responses.

Results. ACh caused ~90% relaxation of U46619-contracted aorta from sham-exposed mice irrespective of viral infection and ebselen treatment (n=6). However, CS-exposed mice had significantly impaired aortic relaxant responses to ACh (n=8, ~50 % R_{max}, p<0.0001), which was further impaired following Mem71 infection (n=8, ~35% R_{max}, p<0.01). Remarkably, ACh prompted a 90% relaxation in CS+ebselen treated mice irrespective of viral infection (n=7, p<0.0001). SNP caused ~90% maximum relaxation in all aortae, irrespective of CS status, viral infection or ebselen treatment.

Discussion. CS exposure caused significant aortic endothelial dysfunction which was further deteriorated after viral infection. Ebselen prevented the CS-induced endothelial dysfunction irrespective of viral exacerbation, suggesting this novel therapeutic may be crucial in the treatment of cardiovascular comorbidities seen in AECOPD.

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Differential activation of human MRGPRX2 by polymyxins and related antibiotics

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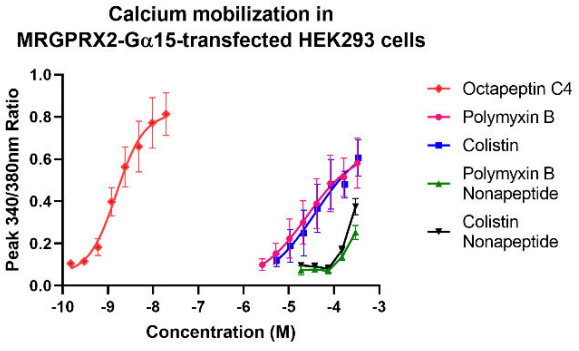
Introduction. The emergence of drug-resistant Gram-negative bacteria has led to re-interest in polymyxin B and colistin and novel polymyxin analogues (e.g. nonapeptides and octapeptin) for improved antimicrobial activity. Polymyxins have been previously reported to trigger mast cell activation through an IgE-independent mechanism, with a recent study demonstrating this to be Mas-related G protein-coupled receptor X2 (MRGPRX2)-dependent (Zhan et al., 2019).

Aims. To characterise the ability of novel polymyxin-based antibiotics to activate human mast cells via MRGPRX2.

Methods. We used a HEK293 cell line expressing human MRGPRX2 and the G protein Ga15 and the human mast cell line LAD2 that natively expresses MRGPRX2. Calcium mobilization in response to the polymyxins was measured using Fura2. Degranulation of LAD2 cells was quantified by release of β-hexosaminidase.

Results. Octapeptin C4, polymyxin B and colistin triggered calcium mobilization in MRGPRX2-transfected HEK293 cells with the former being highly potent (Fig 1). No response was observed in non-transfected cells. A similar pattern was observed in LAD2 cells in both Ca^{2+} mobilisation and degranulation studies. In contrast, polymyxin nonapeptides were far less potent mobilizers of Ca^{2+} (Fig 1) and failed to induce degranulation in LAD2 cells.



Discussion. Activation of human mast cells by the polymyxins and octapeptin was MRGPRX2-dependent with octapeptin C4 being one of the most potent activators of MRGPRX2 yet described. Thus, compared to the clinically used polymyxins (B and colistin), octapeptin might be more liable to trigger hypersensitivity whilst nonapeptide polymyxins might be less likely to trigger such adverse events. The molecular mechanisms underpinning these differences in polymyxin activation of MRGPRX2 require further investigation to facilitate the discovery of new-generation safer polymyxins.

Ref: *Zhan et al (2019) Chem Biol Interact 308:304-311.*

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The effect of substance P and its common metabolites on MRGPRX2 activation in human mast cells

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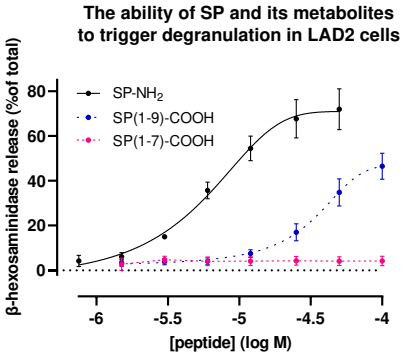
Introduction. As well as binding to the neurokinin 1 receptor (NK_1R), substance P (SP, sequence: RPKPQQFFGLM-NH_2) has been shown to activate the Mas-related G protein-coupled receptor X2 (MRGPRX2) receptor on mast cells (MCs), triggering degranulation with the release of inflammatory mediators such as histamine. SP undergoes rapid C-terminal truncation *in vivo* to generate the major metabolites SP(1-9)-COOH and SP(1-7)-COOH. While the C-terminus of SP has been shown to be critical for NK_1R activation, we predicted that the polybasic N-terminus of SP would be key for MRGPRX2 activation.

Aim. To determine if the major metabolites of SP, SP(1-9)-COOH and SP(1-7)-COOH retained activity at MRGPRX2.

Methods. SP-NH_2, SP(1-9)-COOH and SP(1-7)-COOH were synthesized by solid-phase peptide synthesis and purified to >95% purity by HPLC. Stably transfected HEK293 cells expressing NK_1R or MRGPRX2 and the LAD2 human MC line were used to determine the activity of SP and its metabolites in Ca^{2+} mobilization (Fura2), degranulation (β-hexosaminidase release) and cytokine (CCL2 release) assays.



Results. As expected from prior studies, both metabolites had essentially no activity at NK_1R, even at very high concentrations. In contrast, although reduced in comparison to SP, SP(1-9)-COOH remained able to activate MRGPRX2 when measured by Ca^{2+} mobilization, MC degranulation (Fig 1) and cytokine production. SP(1-7)-COOH however, only retained weak cytokine release activity.

Discussion. Here we show that despite having no activity at NK_1R, the major *in vivo* metabolite of SP, SP(1-9)-COOH, is still able to activate MRGPRX2, with reduced potency compared to intact SP. This suggests that SP(1-9)-COOH, in particular, may play a regulatory role through modulation of MRGPRX2. However, given the relatively low potency of both SP and SP(1-9)-COOH at MRGPRX2, the *in vivo* relevance of this finding requires further examination.

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Cigarette smoking does not worsen skeletal muscle contractile function or loss caused by acute viral infection in mice

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Introduction. Chronic obstructive pulmonary disease (COPD) is characterised by progressive airflow limitation that is largely attributed to cigarette smoking (CS). Skeletal muscle wasting is a prevalent comorbidity that affects up to 40% of COPD patients. Muscle wasting is most frequently reported following an episode of viral-induced acute exacerbation of COPD (AECOPD), which may prolong hospital stay and lead to future readmission. However, the underlying mechanisms responsible remain poorly defined.

Aims. To investigate whether viral infection per se causes muscle wasting and dysfunction *in vivo*, and if so, determine whether such an effect would be amplified by chronic CS exposure.

Methods. Male BALB/c mice were exposed to either room air (sham) or CS (9 cigarettes per day, 5 days per week) for 8 weeks followed by inoculation with either influenza A virus (IAV; Mem71, $1x10^{4.5}$ PFU) or diluent (PBS) and culled 3 days post-infection. Muscle function tests were performed, and prime mover muscles of the hind limbs were collected for morphological analyses.

Results. IAV infection resulted in no change in tibialis anterior (TA) muscle mass, despite marked lung inflammation evidenced by a 11.6-fold increase in bronchoalveolar lavage fluid cellularity (p<0.001 vs sham diluent, n=10). CS exposure alone induced a 13% loss in TA muscle mass (p<0.001 vs sham diluent; n=10). When CS exposure was combined with IAV infection, lung inflammation was exacerbated 2-fold (p<0.001 vs CS diluent), however, no further reduction in TA muscle mass was observed (p=0.99 vs CS diluent). Despite the unchanged muscle mass, the strength of TA was reduced by 52% by IAV infection (p<0.001 vs sham diluent, n=6) which was not further compromised by CS exposure (p=0.20 vs sham IAV, n=6).

Discussion. Acute IAV infection per se specifically impaired muscle function without muscle loss. This suggests that muscle function may be more vulnerable to IAV infection than muscle mass. The lack of an additive effect may imply the involvement of mechanisms other than simple lung inflammation in driving the observed muscle dysfunction.

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Expression profiling of specialised pro-resolving receptors in human peripheral blood mononuclear cells (PBMCs) and polymorphonuclear leukocytes (PMNs)

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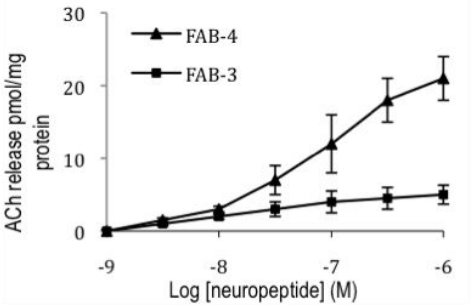
Introduction. Resolving excessive inflammation and preventing its progression into chronic pathophysiology such as autoimmune diseases is heavily regulated by the actions of specialised pro-resolving mediators (SPMs) and their six cognate G protein-coupled receptors (GPCRs) (Krishnamoorthy et al, 2018). SPM and the SPM-GPCRs exert their effects on PBMCs and PMNs to ultimately drive resolution of inflammation (Serhan & Levy, 2018).



Aims. We aim to construct a comprehensive profiling of the surface protein and gene expression patterns of the six SPM-GPCRs in human PBMCs and PMNs of healthy donors and compare with that of autoimmune patients, to investigate the association between the expression patterns of the receptors and the pathology of the diseases.

Methods. Human PBMCs and PMNs were isolated from whole blood of healthy donors and their surface protein and gene expression of the six SPM-GPCRs were assessed through flow cytometry and quantitative polymerase chain reaction (qPCR), respectively.

Results. Robust surface expression of SPM-GPCRs was observed on both PBMCs and PMNs as supported by the gene expression analysis. However interestingly, the pattern of surface and gene expression did not correlate.

Discussion. Our findings indicate that human PBMCs and PMNs from healthy donors readily express the six SPM-GPCRs and we have established the methods to assess the expression profile in these cells. These results will be compared to that of autoimmune disorder patients, and provide insight on the pathology of the disease and identify potential drug target(s).

Krishnamoorthy N et al (2018) Physiol Rev 98, 1335-1370
Serhan C & Levy B (2018) J Clin Invest 128, 2657-2669

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Characterisation of chemokine-dependent signalling in monocytes via chemokine GPCRs CCR1 and CCR2

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Introduction. Chemokines are chemoattractant cytokines which interact with G protein-coupled receptors expressed on the surface of leukocytes. They are key regulators of monocyte recruitment, a crucial step in regulation of inflammatory responses at the sites of inflammation. CC chemokine receptors CCR1 and CCR2 are abundantly expressed on monocytes and are potential therapeutic targets in inflammatory diseases including atherosclerosis and rheumatoid arthritis. Blockade of these receptors has been effective in animal models but has failed in clinical trials due to lack of efficacy. A major reason for the failure is the complexity in the chemokine-receptor signalling network.

Aim. To globally characterise the signalling network downstream of activation of robustly activated chemokine receptors in monocytes using signalling assays and phosphoproteomics.

Methods. We carried out well-established cell -signalling assays for chemokines receptors, including chemotaxis (96- well MultiScreen plates, Merck) and ERK phosphorylation (AlphaLISA Surefire Ultra, Perkin Elmer) in monocyte-like THP-1 cells to characterise CCR1- and CCR2- dependent downstream effects after activation with their specific chemokines. We will perform a phosphoproteomics study using data-independent acquisition (DIA) mass spectrometry to quantify changes in phosphopeptide between untreated and CCL5-stimulated THP-1 cells expressing CCR1.

Results. The chemokine CCL5 elicited a concentration-dependent increase in chemotaxis of THP-1 cells with a classical bell-shaped curve and a peak concentration of 10nmol/L. CCL5 elicited robust pERK signalling with EC_{50} of 1.42 nmol/L. CCL5-dependent signalling was completely inhibited by CCR1 antagonist BX471 (1µmol/L). This was previously performed for CCR2. CCL2 stimulation time course in phosphoproteomics study promoted 460 phosphosites, 329 unique proteins (1-way ANOVA with FDR <0.05) including phosphorylation of CCR1.

Discussion. These findings will provide critical insights into complexity of chemokine receptor signalling cascade. The validation of targeted proteins identified in the chemokine-dependent signalling pathway will result in development of novel therapeutic interventions for overall regulation of monocyte recruitment.

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Involvement of P-glycoprotein (ABCB1) in the export of amyloid-beta from the Alzheimer's brain

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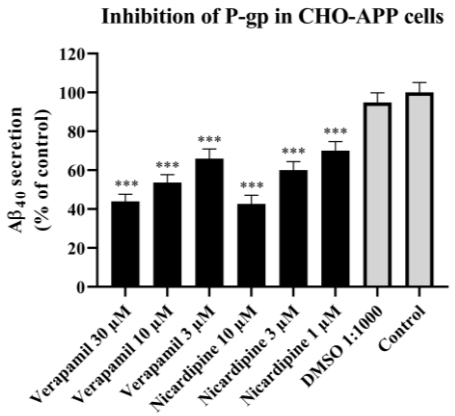
Introduction. Defective clearance mechanisms in the Alzheimer's brain lead to the accumulation of amyloid-β (Aβ) peptides, in particular $Aβ_{40}$ and $Aβ_{42}$. These peptides form characteristic plaques and toxic soluble oligomers that contribute to impaired synaptic function, memory loss, neurodegeneration, and cognitive decline. The role of P-glycoprotein (P-gp or ABCB1) in exporting Aβ across the blood brain barrier is well-established, however little is known about how these hydrophobic peptides, initially produced in neurons, are first released into the extracellular space.



Aims. To assess whether P-gp is expressed, active, and involved in the export of Aβ from neurons.

Methods. We measured P-gp protein expression and activity using Western blotting and calcein-AM assays respectively. CHO-APP and SK-N-SH cells were treated with the P-gp inhibitors verapamil and nicardipine, then $Aβ_{40}$ and $Aβ_{42}$ secretion into cell media was quantified by ELISA.

Results. P-gp protein expression was detected in human neuroblastoma cell lines and primary rodent brain derived neurons. P-gp was shown to be active in SK-N-SH cells, using the Calcein-AM assay. Chemical inhibition of P-gp reduced export of $Aβ_{40}$ and $Aβ_{42}$ from CHO-APP and SK-N-SH cells in a concentration-dependent manner.

Discussion. Data show that Aβ can be transported by P-gp, and inhibition of P-gp impairs export of these peptides out of neurons. These results present a paradigm shift in thinking of what drives export of these peptides from neurons and what impact a reduction in P-gp over time may have on markers of neurodegeneration or Alzheimer's symptoms. Discerning the cellular clearance mechanisms of Aβ will provide significant contributions to our understanding of the pathophysiology and treatment of Alzheimer's disease.

Chai AB, Leung GK, Callaghan R, Gelissen IC (2020) *FEBS J* 287(4):612-625

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Utilizing mini-G protein biosensors and BRET to profile orexin receptor pharmacology

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Introduction. The orexins, orexin A (OxA) and orexin B (OxB), are peptide agonists that bind to orexin receptor 1 (OxR1) and orexin receptor 2 (OxR2). OxA binds to both receptors with similar affinity while OxB exhibits substantially decreased affinity for OxR1. The orexin receptors have been reported to exhibit diverse G protein coupling behaviour that is tissue and cell-dependent. As such, characterization of the coupling capabilities of the receptors has remained somewhat controversial due to the large variability in observations dependent upon experimental variables.

Aims. We aimed to investigate the G protein activation profiles of the orexin system using mini-G protein biosensors in HEK293FT cells.

Methods. We utilized cutting-edge bioluminescence resonance energy transfer (BRET) technologies along with the newly developed G protein activation biosensors known as mini-G proteins (Wan et al, 2018) to monitor biosensor recruitment to activating GPCRs within live HEK293FT cells in real time.

Results. Mini-G protein recruitment was successfully monitored to both orexin receptors upon stimulation with either OxA or OxB using BRET. Both receptors coupled to multiple mini-G proteins with the most robust recruitment occurring with the receptors' prototypical G protein G_q (mGsq mini-G protein). Divergences in the strength of mini-G protein recruitment was observed between the receptors but also between OxA and OxB stimulation indicating ligand-dependent effects on mini-G protein recruitment.

Discussion. These findings demonstrate that the orexins exhibit the capacity for diverse G protein interactions within HEK293FT cells as demonstrated with the use of mini-G protein biosensors. Mini-G protein biosensors present a powerful tool to investigate the signalling capabilities of GPCRs.

Wan, Okashah, Inoue et al (2018) J Biol Chem 293:7466-7473

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Ivacaftor, a cystic fibrosis modulator drug, in pregnancy and lactation: entry into the developing brain and lung

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Introduction. With the rapid expansion of therapeutic modulators of cystic fibrosis transmembrane conductance regulator (including ivacaftor), the life expectancy of cystic fibrosis (CF) patients has increased substantially, enabling more CF women to reach child-bearing age (Heltshe et al, 2017). However, it is uncertain how safe is long-term use of ivacaftor during pregnancy and breastfeeding for the fetus and newborn, especially for their developing brain.

Aims. 1) To determine transfer of ivacaftor across placenta in pregnancy and breast tissue during lactation. 2) To investigate entry of ivacaftor into brain and lungs of pre- and postnatal rats following acute or long-term exposure.

Methods. Pregnant Sprague Dawley rats at embryonic day (E) 19 were administered a single ip injection of ivacaftor at a clinically relevant dose of 40 mg/kg traced with [^{3}H] ivacaftor. Following terminal anaesthesia (ip urethane 2.5 g/kg), blood samples from individual pups were serially collected along with time-matched maternal blood samples and radioactivity was measured by liquid scintillation counting. Postnatal animals were either administered a single ip injection of radiolabelled ivacaftor or exposed to the drug via milk from an orally treated dam over 4-14 days. Blood, brain and lung samples were analysed using liquid scintillation or LC-MS.

Results. 1) Placental transfer of ivacaftor from maternal to fetal plasma was around 30%. In lactating dams, the average concentration of ivacaftor in pup plasma was about 40% of the maternal plasma concentration. 2) In acutely exposed postnatal pups, the average tissue/plasma concentration ratios were around 2% and 330% for brain and lungs respectively. Following 4 days of exposure, the ratios increased in the brain to over 20% but decreased in lungs to about 180%. With longer exposure these ratios decreased in all tissues by day 14.

Discussion. Fetal and postnal rats are exposed to maternally administered ivacaftor via placental and milk transfer. Entry of ivacaftor into the brain was much lower than into lungs at all ages. These data may suggest the possibility of babies facing therapeutic level of ivacaftor that preferentially enters their lungs. Further investigation is required to determine the exact mechanism that allows for entry of ivacaftor into the brain and lungs at different developmental ages, and the effects of ivacaftor in the developing human brain.

Heltshe SL et al (2017) J Cyst Fibros 16: 687-694

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Can monitoring clozapine levels reduce the occurrence of peripheral ADRs?

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Introduction. Clozapine is the most effective antipsychotic for treatment-refractory schizophrenia for reducing positive psychotic symptoms. It has a lower rate of drug discontinuation compared to other second-generation antipsychotics and is associated with a reduction in overall mortality. In spite of this, clozapine remains underutilized due to adverse drug reactions (ADRs) such as weight gain, hypersalivation and constipation. Individual ADRs may be associated with clozapine levels. Information on the relationship between clozapine levels and ADRs can help inform clinicians and patients in making choices about optimising clozapine dose to balance therapeutic effect and ADRs.

Aims. The aim of this systematic review is to determine the correlation between clozapine levels and ADRs, and to undertake pairwise meta-analyses, where possible.

Methods. Studies were searched from four electronic databases (PubMED, EMBASE, PsycINFO and CINAHL) from inception to 12 June 2020. Studies were included if they had adult patients (≥16 years), provided data on steady-state trough clozapine levels, and reported on clozapine associated ADRs. Pregnant women, case reports and series were excluded.

Results. A statistically significant correlation was found for clozapine serum levels and triglycerides (n=70; r=0.303, 95% CI 0.0119 to 0.546, p=0.042, I^2=22.9%), heart rate (n=137; r=0.269, 95% CI 0.0918 to 0.486, p=0.035, I^2=58.4%), and overall combined ADRs (n=160; r=0.264, 95% CI 0.110 to 0.405, p=0.001, I^2=0%), but not for absolute neutrophil count (n=223; r=-0.164, 95% CI -0.529 to 0.253, p=0.444, I^2=86.3%) or total white cell count (n=189; r=0.0176, 95% CI -0.203 to 0.237, p=0.878, I^2=56.1%). Interestingly, norclozapine serum levels was found to be statistically correlated to triglycerides (n=120; r=0.211, 95% CI 0.0305 to 0.378, p=0.022, I^2=0%), total cholesterol (n=120; r=0.272, 95% CI 0.0948 to 0.432, p=0.003, I^2=0%), and weight gain (n=118; r=0.208, 95% CI 0.0261 to 0.377, p=0.025, I^2=0%).

Discussion. Clozapine levels may be correlated to metabolic abnormalities and increased HR. However, a true correlation can be difficult to interpret clinically using observational studies. Further prospective, randomized studies are needed to identify the cause-effect relationship of clozapine level and peripheral ADRs.

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Entry of valproate and lamotrigine into the developing brain

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Introduction. Pregnancy presents a serious challenge to epilepsy management, with long-term effects on children of epileptic mothers not well characterised. The antiepileptic drug valproate has been flagged for its dose-dependent teratogenicity, including potential deleterious effects on cognition several years after birth (Meador et al, 2013). Nevertheless, it remains in use, often in combination with other drugs such as lamotrigine, as the only means of seizure control for many pregnant women. Current treatment recommendations are based largely on expert opinion of clinicians and retrospective studies of pregnancy registers. Animal studies investigating mechanisms of placental transfer and developmentally regulated brain entry of antiepileptics are lacking and remain essential.

Aims. To determine the role of brain and placental barriers in modulating valproate and lamotrigine entry into the developing central nervous system.

Methods. The transfer of clinically relevant doses of valproate and lamotrigine from the plasma into the brain and cerebrospinal fluid (CSF) was estimated in Sprague-Dawley rats at three developmental stages (embryonic day (E) 19, postnatal day (P) 4 and adult) using intraperitoneal injections of radiolabelled drugs. Placental transfer was estimated at E19 using foetal/maternal plasma concentration ratios.

Results. Both valproate and lamotrigine entered the foetal brain at E19 to a higher level than at either postnatal age, however entry into the CSF was only higher for valproate at E19. The placental barrier provided a higher protection for lamotrigine (foetal/maternal plasma ratio was 20-30%) than valproate (foetal/maternal plasma ratio was 70-80%). At P4, the combination of valproate and lamotrigine had no significant effect on the entry of either drug into the brain.

Discussion. Higher drug entry into foetal circulation, CSF, and brain at E19 may contribute to the increased deleterious effects of valproate during pregnancy. No difference of valproate entry in the combination treatment at P4 indicates that limited risks remain when attempting to use lamotrigine to reduce the necessary valproate dose for seizure control.

Meador KJ et al (2013) Lancet Neurol 12:244-252.

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Male-female differences in the effects of age on mouse activities of daily living measured using an automated behavioural classification

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Introduction. Activities of daily living (ADLs) are fundamental self-care tasks that are routinely performed by individuals, and are important global health outcomes. Independence in ADLs differs with age and sex in humans. Laboratory animals are often used to study pharmacological and toxicological effects. However, the impacts of age, sex and the interaction between these two factors on murine routine activities have not been well characterised.

Aims. Using a mouse model, we aimed to study how age and sex affect murine ADLs over 23 hours.

Methods. Young (2.5 months) and old (21.5 months) C57BL/6 male and female mice were assessed using an automated behavioural recording machine – the Laboratory Animal Behaviour Observation, Registration, and Analysis System for 23 hours. Mice were individually caged for recording of ADLs including distance travelled, mean gait speed, and the durations of locomotion, rearing, climbing, grooming, immobility, eating and drinking.

Results. Compared to young mice of the same sex, old mice travelled significantly shorter distances with slower gait speed and had shorter durations of locomotion, rearing, climbing and immobility, particularly during the dark cycle between 7pm-7am. Compared to old males, old females reared more during the light cycle between 11am-7pm ($p<0.05$). Young female mice spent significantly more time climbing than young males. Significant age-sex interactions were detected for rearing and climbing, in which females did not decline as much in old age as males. Within the same sex, old mice groomed more than young mice ($p<0.05$). No differences were observed with age and sex for the duration of drinking and eating.

Discussion. Our results suggest that in mice, old age may decrease exploratory activities but increase grooming. The age-related decline varies between sexes and tends to be more severe in males. This assessment should be a useful translatable outcome to study how different interventions affect ADLs in rodents of different ages and sexes.

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Self-nanomicellizing solid dispersion of USA612: Solubility improvement

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Introduction. Despite broad-spectrum antimicrobial activities of USA612 including antibacterial, antifungal, and antiviral activities, the translation of USA612 into a useful therapeutic is still limited due to its poor aqueous solubility (≤40 µg/mL). To overcome this formulation challenge and enhance its aqueous solubility, self-nanomicellizing solid dispersion (SNMSD) strategy was used to develop a novel USA612 formulation (NUF).

Aims. Development of NUF using SNMSD strategy for the enhancement of its aqueous solubility.

Methods. Since USA612 does not have a chromophore, a new analytical method was developed using HPLC Refractive Index (RI) detectors. Based on the literature review, three polymers (Soluplus, HPMC- ASLG and HPMC-ASMG) were selected for their potential to improve the solubility with the drug/polymer ratio 1:5. The solvent evaporation technique was used to prepare three SNMSDs. The solubility was evaluated at predetermined time intervals using our developed analytical method. The NUF was characterized using differential scanning calorimetry, X-ray diffraction, scanning electron microscopy and Fourier transform infrared spectroscopy. Besides, its self-micellizing properties were assessed after dissolving in aqueous media for particle size, zeta potential, loading ability, and morphology through transmission electron microscopy analysis.

Results. A simple, reproducible and rapid chromatographic methodology has been developed and applied successfully for the determination of USA612 from aqueous media. The results showed that the linear range for USA612 was 100-500 µg/mL with the squared correlation coefficients (R2) being 0.9992. The optimised NUF showed significantly improved aqueous solubility of >1000 µg/mL in PBS pH-7.4. The amorphization, hydrogen bonding interaction, and micellization could have played a vital role in the improvement of the solubility profile of USA612.

Discussion. The results demonstrated that SNMSD system could serve as a promising strategy to improve USA612 solubility and NUF could be a potential candidate for the treatment of infectious diseases.

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Does co-administering whole and crushed paracetamol with Gloup alter drug dissolution profile *in vitro*?

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Introduction. Gloup is designed to facilitate swallowing of tablets and capsules whole for those who find it difficult. In practice, Gloup is used with crushed tablets as well as whole. We have previously shown that dissolution of crushed tablets co-administered with thickened fluids can be significantly delayed.

Aim. To evaluate the effect of co-administering whole and crushed tablets with Gloup on drug dissolution.

Methods. The dissolution of whole and crushed paracetamol tablets mixed with two IDDSI (International Dysphagia Diet Standardisation Initiative) level 3 swallowing medication lubricants (Gloup Low Sugar, Gloup Original strawberry/banana) was tested in simulated gastric fluid. Comparisons were made with water, and water thickened to IDDSI level 3 (liquidised/moderately thick) using a xanthan gum product (Easythick Advanced).

Results. Dissolution of immediate release paracetamol tablets is very fast whether crushed or whole. When co-administered with Gloup dissolution of whole was slightly delayed but reached 85% by 30 to 60 min. Dissolution of crushed tablets was affected more strongly than whole tablets, as 50-70% was dissolved by 30 minutes, 85% dissolution was reached after 80 to 150 min, and with little difference between Gloup and water thickened with xanthan gum.

Discussion. There is a delay in paracetamol dissolution when co-administered with Gloup instead of water, and this delay is increased if tablets are crushed. However it is important to bear in mind that salivary mixing and shear forces exerted on the Gloup during oral preparation and swallowing are not accounted for by the simple dissolution test, so more research is needed before any conclusions around clinical relevance can be made.

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Co-amorphous kanamycin-amino acid spray-dried inhalable particles: the influence of feed concentration in achieving high aerosolization

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Introduction. Co-amorphization of kanamycin with amino acids by co-spray drying is known to improve aerosolization of kanamycin. However, various spray drying processing factors may influence the aerosol performance of these co-amorphous systems.

Aims. This study aimed to assess the influence of feed concentration on aerosolization for kanamycin-amino acid co-amorphous powders using kanamycin-valine (KV) as a model system.

Methods. Using 0.2% and 0.4% w/v feed solutions, KV02 and KV04 formulations were prepared by spray-drying kanamycin and valine in 1:1 molar ratio, and K02 and K04 by spray-drying kanamycin alone. All formulations were characterized using X-ray diffraction (XRD), Infrared (IR) spectroscopy, Thermogravimetric Analyzer (TGA), Differential Scanning Calorimetry (DSC), and Scanning Electron Microscopy (SEM). The *in-vitro* aerosol performance was assessed using Next Generation Impactor (NGI).

Results. The average particle sizes of the KV02 and KV04 were around 0.9 and 1.2 µm. All co-spray dried particles were amorphous determined by XRD. Further the co-amorphicity of the powders was confirmed using DSC. Although both KV02 and KV04 showed higher ED, improvement in FPF was observed for only KV02. For example, the emitted dose (ED) of the K02 and K04 were 71.1% and 66.9%, and the ED of kanamycin from KV02 and KV04 were 78.8% and 77.5%, respectively. In contrast, the Fine Particle Fraction (FPF) of kanamycin significantly increased for kanamycin-valine formulations at 0.2% feed concentration, e.g., 70% for K02 vs 78% for KV02 ($p<0.05$) while the FPF of kanamycin did not change for formulations at 0.4% feed concentration, e.g., 73% for both K04 and KV04.

Discussion. The similarity in ED and the difference in FPF between KV02 and KV04 suggest that although the co-amorphous powders exit the device, the powders disperse into primary particles and smaller aggregates whose size ultimately define particle deposition behaviour. While further studies are undergoing to reveal the exact mechanism, it seems that aerosolization behaviour is influenced by feed concentration for kanamycin-amino acid co-amorphous systems.

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Characterization of Novel Inulin hydrogels loaded with 5-fluorouracil for colon delivery

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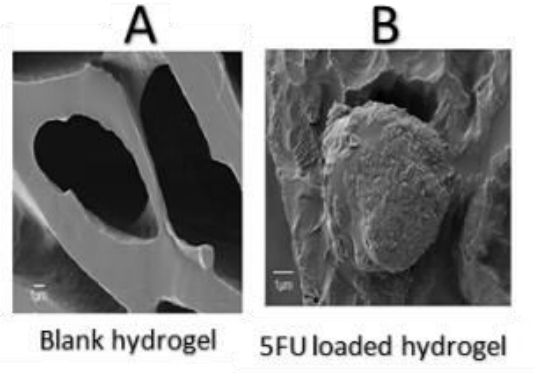
Introduction. A significant challenge in the drug treatment of colon cancer is the optimization of drug delivery as well as how to avoid severe systemic side effects during chemotherapy. For instance, 5-fluorouracil (5-FU) is currently administered by intravenous route. This limits the clinical application of 5-FU due to unwanted systemic side effects and off-target problems. To address this limitation, there is an urgent need for the development of a suitable drug delivery platform which can help to reduce the toxicity of 5-FU by providing targeted, localized and sustained drug release to the colon.



Aims. To characterize smart hydrogels loaded with 5-FU for colon targeted delivery

Methods. Inulin hydrogels were prepared by the esterification reaction by crosslinking raw inulin with pyromellitic dianhydride (PMDA) using triethylamine as a catalyst[1] followed by loading with 5-FU using the swelling method. The physicochemical characterization of the drug-loaded 5-FU gels was determined using different techniques such as Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM), *in-vitro* release, degradation and cytotoxicity studies.

Results/Discussion. FTIR was used to confirmed the encapsulation of 5-FU with new peaks at 3071, 1246, 1433, 750 cm^{-1}. XRD confirmed the disappearance of 5-FU crystalline peaks in the formulation. SEM showed the change in surface morphology and the encapsulation of 5-FU within the pores (Figure 1). HPLC results confirmed encapsulation of 5-FU with loading between 8.2-18.0 % depending on the ratio of PMDA crosslinker. The drug release was characterized by both burst and control release in the pH conditions of the gastrointestinal tract. Furthermore, 5-FU loaded gels are degradable with the use of inulinase and MTT assay shows a dose-dependent efficacy against HCT116 from the 5-FU loaded hydrogels. These preliminary results make the hydrogels a promising platform for the localized delivery of 5-FU to the colon.

1 Afinjuomo, F., et al., Synthesis and characterization of a novel inulin hydrogel crosslinked with pyromellitic dianhydride. Reactive and Functional Polymers, 2019. **134**:

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Glycine-Proline-Glutamate loaded bi ligand niosomal delivery system interactions on Cell Models

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Introduction. Glycine-Proline-Glutamate (GPE) is a neuroprotective peptide with a dose-dependent and receptor mediated mechanism leading to downregulation of inflammation, promotion of astrocytosis, and inhibition of apoptosis, beneficial in many neurodegenerative diseases. Oral and intravenous administration of GPE has limited transport across the blood-brain-barrier (BBB) due to its hydrophilicity and susceptibility to enzymatical degradation in the body. Encapsulation into a bi ligand niosomal delivery system can improve both the stability and penetration of GPE.

Aims. To evaluate the interaction of GPE loaded bi-ligand niosomal delivery system on Rat Brain Endothelial (RBMVE) cells mimicking the BBB.

Methods. The niosomes were fabricated using thin-film hydration technique by mixing surfactant, dicetyl phosphate, poly-l-arginine-conjugated polyethylene glycol and cholesterol dissolved in a mixture of organic solvents. The RI7 ligand conjugate is then incubated with niosomes overnight to form the bi-ligand niosomal delivery system. Cell viability of GPE between 5-1000 µM was determined by MTT assay. Cellular uptake of GPE and its mechanism on Caco-2 cells were also investigated in the absence and presence of the niosomes.

Results. GPE loaded bi-ligand niosomal delivery system was successfully fabricated and evaluated on RBMVE cells, showing no significant cytotoxicity at low concentrations and improved uptake and transport in the presence of the bi-ligand niosomal system.

Discussion. The niosomal delivery system and its individual constituents are not toxic to cells and have improved cellular uptake of the drug delivery system into the cells when compared against free drug suggests this niosomal delivery system has potential for neuropeptide delivery across the BBB.

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Hand sanitizer for hand hygiene during COVID-19 in New Zealand: A response to the community's need by staff and students of the School of Pharmacy, University of Otago

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Introduction: Hand sanitizer is an essential commodity to maintain hand hygiene and to prevent the spread of SARS-CoV-2, the causative virus of COVID-19, as well as other microorganisms. During this COVID-19 pandemic, the demand for hand sanitizer has increased dramatically worldwide, including New Zealand (NZ).

Aims: The aim of this project is to describe how the University of Otago School of Pharmacy contributed to the community need with hand sanitizer during COVID-19.

Methods: The 'Dr Das Research Group' was formed and registered with the Ministry of Primary Industries to prepare hand sanitizer voluntarily at the School of Pharmacy, University of Otago (UoO). This group produced alcohol-based hand sanitizer as prescribed by the World Health Organization, following all health and safety and regulatory guidelines. The product was distributed to essential workers during NZ's lockdown in March-May, 2020 through the University's Emergency Response team.

Results: Starting just three days before the lockdown, and working during the lockdown, 1200 litres of hand sanitizer were produced without any automation. It was used by the essential services of the UoO's Dunedin campus, local Civil Defence, the Dunedin and Canterbury Police, Fire and Emergency, St John Ambulance Service, Driving Miss Daisy Dunedin and a Māori Health provider during lockdown. In addition, Dr Das has been consulted by many health providers in NZ for advice and has inspired many universities, organizations and individuals to produce their own hand sanitizer.

Discussion: The skills necessary for the preparation of hand sanitizer are universal for pharmaceutical scientists giving the opportunity for the School of Pharmacy to make a contribution to society during lockdown. This project had a positive impact on the community by taking pressure off commercial suppliers, freeing up capacity for the general public, and minimizing risk of front line workers. The project has attracted significant media attention locally and internationally.

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Novel functionalized mesoporous silica nanoparticles targeting estrogen receptor over-regulated breast cancer

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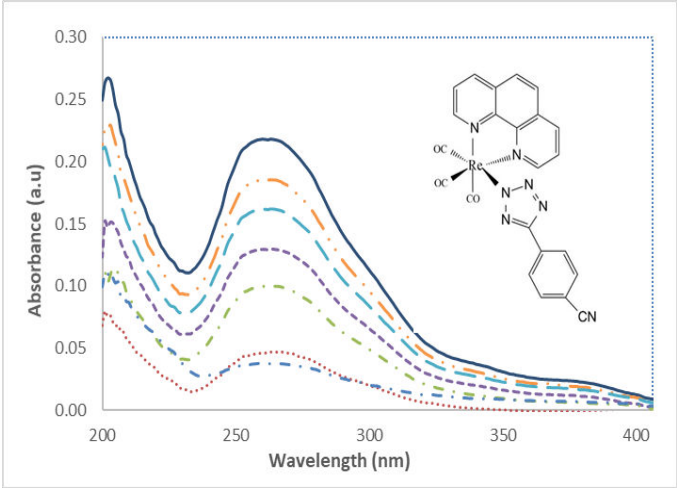
Introduction. Conventional chemotherapy for breast cancer (BC) destroys both healthy and cancerous cells. To address this issue, a novel drug delivery system (DDS) based on mesoporous silica nanoparticles (MSNs) was fabricated to selectively deliver incorporated anticancer drugs to BC. The inorganic fluorophore Rezolve-L1 (REZ-L1) was trialled as an encapsulated material to provide the proof of concept.



Aims. Successfully synthesize and functionalize MSNs with active targeting ligands for enhanced selectivity to BC. REZ-L1 molecules were encapsulated within the mesopores with high capacity. REZ-L1-loaded MSNs achieved active endocytosis into BC cell lines.

Methods. Surface functionalization was conducted via click-chemistry. REZ-L1 molecules were loaded on to MSNs used solvent immersion technique. Encapsulation and loading capacity were determined via UV-Vis, from varied concentrations of REZ-L1 solutions determined through the Beer Lambert equation.

Results. The system achieved a grafting ratio of functionalities of approximately 29%. Rezolve-L1 was successfully loaded with a maximal encapsulation efficacy pf 65% and a REZ-L1 loading capacity ranging from 10 to 40% (w/w).

Discussion. The functionalized MSNs were successfully designed and developed for the active targeted delivery of REZ-L1 to BC. This novel DDS offers valuable applications in breast cancer therapeutics and diagnostics

Bader C et al (2014), RSC Adv., 4, 16345

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A Novel Rat Cervical Lymph Cannulation Method to Evaluate the Lymphatic Clearance of Therapeutics from the Brain

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Introduction. Several recent studies have demonstrated the drainage of fluid, immune cells, fluorescent tracers and dyes along meningeal and cervical lymphatic vessels following injection into the brain. However, no previous studies have evaluated the potential role of the lymphatics in the clearance of therapeutics from the brain.

Aim. 1) To develop a new method to cannulate and collect cervical lymph in rats. 2) To determine the lymphatic clearance of therapeutics from the brain in rats.

Methods. Sprague-Dawley rats were anaesthetised with 1.5-5% inhaled isoflurane then cannulated/ligated at the carotid artery +/- at the cervical lymph duct. Rats were administered via direct injection into the brain parenchyma with ^{14}C-ibuprofen (a small molecule, non-lipophilic drug) or ^{3}H-albumin (a model for large protein therapeutics) at a rate of 0.5µl/min over 16 min. Blood and lymph samples were collected for up to 8 h following dosing and brain tissue was collected at 8 h. Samples were subsequently analysed for radioactivity levels via scintillation counting.

Results and Discussion. In rats administered ^{14}C-ibuprofen into the brain, plasma concentrations of ^{14}C-ibuprofen over time were higher in lymph-ligated rats than in lymph-intact rats (plasma AUC 6.0 ± 0.6 versus 2.4 ± 0.7 %dose.h/ml, respectively). This suggests that ibuprofen is cleared from the brain across the blood-brain barrier and has minimal lymphatic clearance.

Following injection of ^{3}H-albumin into the brain, lymph:plasma concentration ratios of ^{3}H-albumin were very high (up to 54:1). Plasma concentrations over time were not significantly different between lymph-intact and lymph-ligated groups, but were lower in lymph-cannulated rats (plasma AUC 3.3 ± 1.0, 3.1 ± 0.9 and 1.1 ± 0.5 %dose.h/ml, respectively). Together these results indicate that albumin is transported from the brain via the lymphatics.

Conclusion. A cervical lymph cannulation method has been developed for the first time in rats. The lymphatics contribute to the brain clearance of the protein albumin, but not for the small molecule therapeutic ibuprofen. Future studies will evaluate whether centrally-acting drugs also undergo lymphatic elimination from the brain as this has the potential to significantly influence drug accumulation in the brain and thus drug efficacy and toxicity.

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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 mechanism 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. Receptor signalling was measured using cAMP accumulation and Ca^{2+} mobilisation assays in the presence of various inhibitors: adenylyl cyclase (2',3'-dideoxyadenosine), $G\alpha_{i/o}$ (pertussis toxin), $G\beta\gamma$ (gallein), protein kinase A (KT5720), exchange protein activated by cAMP (ESI-09), protein kinase C (GF109203X), and Ca^{2+} chelator (BAPTA-AM). 3D cellular invasion was assessed using microscopy.

Results. Preliminary screening identified three TNBC cell lines which possess elevated cAMP and increased intracellular Ca^{2+} in response to β_2-adrenoceptor stimulation by formoterol; MDA-MB-453 (pEC_{50} cAMP 8.42 ± 0.25 , Ca^{2+} 7.74 ± 0.27), HCC1806 (pEC_{50} cAMP 8.39 ± 0.04, Ca^{2+} 8.70 ± 0.89), HCC1395 (pEC_{50} cAMP 7.97 ± 0.23, Ca^{2+} 8.76 ± 0.51). These results provide preliminary evidence for a cAMP/Ca^{2+} feedforward loop mechanism within these cell lines. Inhibitors were used to delineate any interaction between the cAMP and Ca^{2+} signalling pathways, and to confirm whether activation of the cAMP/Ca^{2+} feedforward loop was required to accelerate invasion.

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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An effective HPTLC method for quality standardisation of Australian propolis

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Introduction. The quality standard of Australian propolis is lacking, which limits the development of the Australian authenticate propolis.

Aims. the aim of this study is to develop an effective method and compare propolis from various sources to determine the variation of chemical profile of Australian propolis.

Methods. Several propolis products and raw materials were collected from various regions including Chinese, New Zealand, Brazilian, and Australia. The raw materials were extracted with ethanol and products diluted with ethanol and applied to HPTLC together with reference compounds. The mobile phase was consisted of toluene-ethyl acetate-formic acid. The chemical profile with or without anisaldehyde - sulfuric acid colour derivatisation was observed by UV light of 254 and 366 nm wavelength to detect the phenolic acids and flavonoids for UV absorbance and fluorescent.

Results. Phenolic compounds and flavonoids showed reproducible different colours under different chromatographic conditions, and samples from different had a similar pattern. Fig 1 was a HPTLC chromatograms of ethanolic extracts of propolis from different sources, under UV 366 nm after derivatisation and showed Australian propolis and Brazilian propolis had multiple yellow bands, while Chinese propolis had multiple light blue bands. Products in Australian market labelled Australian propolis were like Chinese propolis, and one raw Chinese material was adulterated by quercetin.

Discussion. The results indicate HPTLC method is rapid, very informative and efficient in comparing the fingerprint of propolis sources. Further work to collect sufficient representative Australian samples are warranted to determine the variation and quality standards of Australian propolis.

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The expression and function of fatty acid-binding proteins in microglia: what potential role do they play in the uptake of docosahexaenoic acid?

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Introduction. The overactivation of microglia leads to the excessive release of proinflammatory mediators, causing prolonged neuroinflammation, which is detrimental to brain health. Docosahexaenoic acid (DHA) has been shown to alleviate neuroinflammation by inhibiting the release of proinflammatory mediators from microglia. Therefore, the uptake of DHA into microglia is essential for reducing neuroinflammation. Cytoplasmic carrier proteins, fatty acid-binding proteins (FABPs), are involved in DHA trafficking in other cell types.

Aims. This study focused on screening whether various FABP isoforms are expressed in microglia, and whether they are involved in the uptake of DHA into microglia.

Methods. Using immortalised mouse microglia (BV-2) cells, quantitative reverse-transcriptase real-time polymerase chain reaction and western blotting were used to quantitatively determine the mRNA and protein levels of the 10 known FABP isoforms.

Results. FABP3, FABP4, and FABP5 were expressed at both the mRNA and protein level in BV-2 cells. A genetic knockdown approached was then taken to investigate the involvement of these highly expressed FABP isoforms in the microglial uptake of DHA-d5. Interestingly, following 77.5-92.3% (at mRNA level) and 45.4-81.7% (at protein level) knockdown of FABP3, FABP4, and FABP5 at 48 hours, no changes in DHA-d5 uptake into microglia was observed at 2 min.

Discussion. This suggested the involvement of other microglial DHA uptake mechanisms, such as the possible involvement of membrane transporters like fatty acid transport proteins (FATPs).

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Copper complexes modulate the expression and function of P-glycoprotein at the blood-brain barrier

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Introduction. Efflux transporters expressed on the luminal surface of brain endothelial cells act as biochemical barriers to xenobiotic insult and regulate the transport of molecules across the blood-brain barrier (BBB). P-glycoprotein (P-gp) is one of the main efflux transporters involved in the hindrance to central nervous system (CNS) drug delivery. P-gp also plays a major role in the transport of endogenous molecules such as amyloid beta (Aβ) from the brain into the systemic circulation. The expression of P-gp is decreased in people with Alzheimer's disease (AD) which is suspected to decrease the clearance of neurotoxic Aβ from the brain parenchyma. Biometals such as copper (Cu^{2+}), have been shown to be important for the regulation of many signalling pathways in neurons and these pathways are linked to P-gp expression. However, whether Cu^{2+} or other biometals are involved in the regulation of P-gp is yet unknown.

Aims. To increase brain endothelial levels of Cu^{2+} through the use of bis(thiosemicarbazone) (BTSC) complexes, Cu(ATSM) and Cu(GTSM), and assess the impact on P-gp expression and function at the BBB.

Methods. Expression of P-gp at the protein level and transcript level (mdr1) in immortalised human brain endothelial (hCMEC/D3) cells were quantified by Western blot and quantitative polymerase chain reaction respectively following treatment with 25 – 250 nM range of Cu(BTSC) for 24 and 48 h. P-gp function was assessed through the uptake of a fluorescent P-gp substrate, rhodamine 123. Intracellular Cu^{2+} levels were quantified following treatment with the Cu(BTSC)s by inductively coupled plasma mass spectrometry.

Results. Relative to control, Cu(ATSM) significantly enhanced P-gp protein expression 2.0-fold, mdr1 expression 1.5-fold and P-gp function by 29.2% at the 100 nM concentration. In contrast, a 48 h treatment with Cu(GTSM) diminished P-gp expression at both protein (0.5-fold) and mRNA level (0.6-fold) leading to a reduction in P-gp function by 105.4%. Both Cu(ATSM) and Cu(GTSM) were found to increase cytosolic Cu^{2+}.

Discussion. The upregulation of P-gp expression and function was unexpected as Cu(GTSM) was thought to release Cu^{2+} whereas Cu(ATSM) has previously been shown to only release Cu^{2+} under hypoxic conditions. Thus, it may indicate that P-gp expression is mediated by a Cu^{2+} independent mechanism which requires further investigation.

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Method Development and Validation for Determination of Sucralfate in Marketed tablets by HPLC-ELSD

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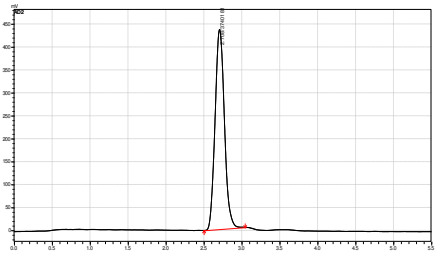
Introduction. The currently reported analytical methods for sucralfate include High-performance liquid chromatography with a Refractive Index detector (HPLC-RI)[1] and HPLC-UV[2], but there is no publication of a chromatographic method with an HPLC using evaporative light scattering detector (HPLC-ELSD) for the quantitative determination of sucralfate. Compared to RI and UV detection, ELSD is less sensitive to temperature and flow-rate variability, has a higher order of magnitude and the response is independent of solvents[3]. These advantages make ELSD a promising detector in the application of determination of sucralfate. Therefore, we developed and validated a simple, rapid, sensitive, and precise HPLC-ELSD method for the determination of sucralfate under the requirement of the ICH Guideline[4].



Method. An analytical method with an HPLC-ELSD was established and validated to determine sucralfate. The method was performed on a column of Luna® 100Å NH_2(4.6 mm × 250 mm, 3 µm) with a mobile phase composed of 0.1% trifluoroacetic acid and acetonitrile in the ratio of 50:50 (v/v) which was run at a flow rate of 0.7 ml/min and the column temperature at 35 ℃, detected by ELSD at a temperature of 55 ℃. The retention time of sucralfate was 2.7±0.5 min. Linearity was acquired in the range of 500-2300 µg/ml. Results. The method was validated for accuracy, precision, repeatability, and robustness. HPLC chromatogram of sucralfate standard reference solution is attached. Assay of sucralfate tablets(Carafate, Australia) was carried out with 96.02 ± 6.33% of the labeled dose observed. Discussion. The proposed method is accurate, precise, and reproducible for the quantitative determination of sucralfate and was easy to apply to the assay of commercially available sucralfate tablets.

1. SP42-NF37 2S (2019).
2. Ahmed S et al (2015) Int J Pharm Sci Rev Res 6(5): 2133-2139.
3. Swartz M (2010) J Liq Chromatogr Relat Technol 33(9-12): 1130-1150.
4. ICH Guideline (2005), Validation of analytical procedures Q2 (R1)

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Determination of residual N, N-dimethylformamide in drug eluting pharmaceutical formulations

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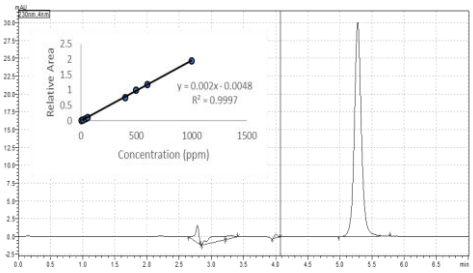

Introduction. The global pharmaceutical market for N,N-Dimethylformamide (DMF) currently exceeds $90 million and expected to increase by more than 14% in the next 7 years. Its high dissolving powers has made it the solvent of choice in many pharmaceutical processes including synthesis, purification and crystallization. However, DMF is a class 2 residual solvent which is considered potentially hepatotoxic. Its concentration in final pharmaceutical products are restricted by regulatory bodies to 880 ppm and a permissible daily exposure of 8.8 mg/day. Although gas chromatography (GC) is a common practice for determination of residual solvents, GC method described in USP does not detect the limit concentrations of DMF.

Aims. Development of a simple, sensitive and selective HPLC method for the determination of residual DMF in anti-cancer drug eluting stents.

Methods. The mobile phase was composed of 50 mM NaH_2PO_4: Acetonitrile (95:5, v/v) and pH was adjusted to 6.5 using NaOH. A flow rate of 1 mL/min was applied, and detection carried out at 230 nm. Elution was performed with C8 column.

Results. The developed method demonstrated linearity at a range of 5-1000 ppm with an accuracy of 98.82 ± 1.79%(r^2=0.9997). Limit of detection and limit of quantitation were determined as 2 and 4 ppm, respectively.

Discussion. The developed method offers a wider linearity range compared to reported methods and a short run time (R_t=5.3 min). This method has been applied for the determination of residual DMF in anti-cancer drug eluting stents, consequently, selectivity was evaluated in the presence of solvents and release media used in formulation processes such as phosphate-buffered saline, tetrahydrofuran, methanol, isopropyl alcohol and dimethylacetamide. The obtained results were within acceptable limits indicating the potential of the method to be applied for other pharmaceutical formulations.

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Pharmacoepidemiology of metaraminol in critically ill patients with shock

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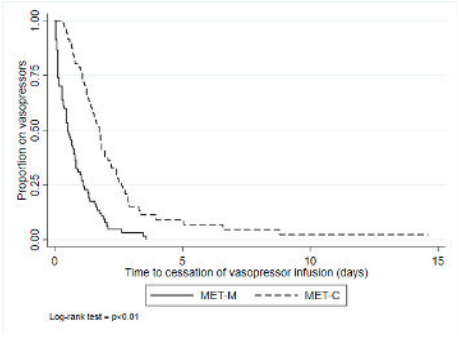
Introduction. The lack of evidence on metaraminol use for the treatment of shock in evidence-based consensus guidelines has not limited its use in critical care. Data from multicentre randomised controlled trials and prospective observational studies have shown metaraminol is utilised in up to 42% of patients in shock.

Aims. To describe the pharmacoepidemiology of metaraminol use in critically ill patients with shock.

Methods. A retrospective observational study was conducted in a 54-bed intensive care unit in Australia. Patients admitted between October 2018 and October 2019 who received metaraminol infusions for the management of shock were included.



Results. A total of 152 patients were included. When metaraminol was used, it was the most common first-line vasopressor started for the management of shock (97%, n=147) and was used as monotherapy in 53% (n=81) of patients. The median duration of metaraminol infusion was 7 h (IQR 3 to 19) and the maximum metaraminol infusion rate used was 4.0 mg/h (IQR 2.5 to 6.0). Peripheral vasopressor infusions were utilised in 96% (n=146/152) of patients. In all of these cases, the peripheral vasopressor used was metaraminol (100%, n = 143/143). Patients were switched from metaraminol to noradrenaline infusions after the insertion of a central venous catheter (R^2=0.89). Patients treated with metaraminol monotherapy (MET-M) had a lower APACHE III score (58 vs 68; p<0.01), a longer duration of metaraminol infusion (12 versus 5 h; p < 0.01), and a shorter duration of overall vasopressor use (12 vs 39 h; p <0.01), compared to those treated with combination vasopressors (MET-C).

Discussion. Metaraminol is often administered as a first-line peripheral vasopressor and is used as a single agent in patients with a lower severity of shock. Metaraminol is commonly transitioned to noradrenaline after the insertion of a CVC.

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Tools to evaluate medication management for caregivers of people with dementia: a systematic review

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Introduction. Caregivers often undertake medication management for people with dementia without formal training. There is a need to evaluate medication management practices for caregivers of people with dementia to identify and address the complexities of medication management.

Aims. To identify tools that evaluate medication management for caregivers of people with dementia and appraise caregiver's involvement in aspects of medication management.

Methods. Database search was conducted in Medline, Embase, PsycINFO, Scopus and International Pharmaceutical Abstracts. Original studies written in English which included tools that evaluated aspects of medication management for caregivers of people with dementia were included. Medication management was defined as the selection, supply, monitoring/review and administration of medications.

Results. A total of 10 studies were included. Medication selection was assessed in six studies, supply and monitoring/review was captured in seven studies, with administration assessed in nine studies. Caregivers were commonly involved in decision-making for medication changes (77.1-86.8%), and in the ordering (55.9-86.0%) and collection (87.0-92.4%) of medications. Tools reported on medication monitoring/review through evaluating caregivers' ability to recognise adverse effects and understanding of when to contact medical providers regarding medication management for the person with dementia. Reported caregiver involvement in medication administration ranged widely (44-94.7%) between tools. Common challenges in medication administration were due to polypharmacy and dosage regimen complexity.

Discussion. Current tools capture specific aspects of medication management, with medication administration the most evaluated aspect of medication management. Future research is needed to develop a tool to holistically evaluate the complexities of medication management for caregivers of people living with dementia to minimise adverse events and reduce caregiver burden.

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Vasopressor dose equivalence: a scoping review and suggested formula.

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Introduction. The calculation of equipotent doses between vasopressor agents is necessary in clinical practice and research pertaining to the management of shock. This is done via calculation of norepinephrine equivalent (NE) doses based on conversion ratios between vasopressor agents. Currently, there are no evidence-based reviews supporting the conversion ratios used in NE calculations.

Aims. To summarise the conversion ratios between vasopressors that have been derived from the literature and provide a formula for researchers to incorporate into their study designs.

Methods. The databases Medline, Embase and Web of Science were searched from inception to 25th June 2020. Additional papers were obtained through a bibliography search of the retrieved articles. Two investigators independently assessed articles for eligibility. Clinical trials published in the English language in adult patients that compared the potency of at least two intravenous vasopressors (norepinephrine, epinephrine, dopamine, phenylephrine, vasopressin or metaraminol), with regard to an outcome of blood pressure, were selected. Conversion ratios were then grouped according to the vasopressor agent and the study setting and were reported in reference to one unit of norepinephrine. The final ratios were calculated as a weighted mean of the ratios from individual studies.

Results. The database and bibliography searches retrieved 14,762 articles. Of these, 19 articles were included for synthesis. The range of conversion ratios equivalent to one unit of norepinephrine were: epinephrine (0.7-1.4), dopamine (75.2-144.4), metaraminol (8.3), phenylephrine (1.1-16.3), and vasopressin (0.3-0.4). The following formula may be considered for the calculation of NE (all in mcg/kg/min, except vasopressin in units/min): NE = norepinephrine + epinephrine + phenylephrine/10 + dopamine/100 + metaraminol/8 + vasopressin*2.5.

Discussion. Our scoping review provides an evidence-based summary of equipotent ratios for the most common vasopressors used in clinical practice. The formula provided may be considered to calculate NE for future studies in the intensive care unit.

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The prevalence of opioid analgesic use in people with chronic non-cancer pain: systematic review and meta-analysis.

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Introduction. The prevalence of opioid use in people with chronic non-cancer pain is unclear.

Aims. To review studies examining the proportion of people with chronic non-cancer pain who report consuming opioids and characteristics associated with their use.

Methods. We searched databases from inception to 8th February 2020. We included observational studies reporting the proportion of adults with chronic non-cancer pain who used opioid analgesics. Opioids were categorised as weak (e.g. codeine) or strong (e.g. oxycodone). Study risk of bias was assessed, and Grading of Recommendations Assessment, Development and Evaluation provided the overall quality. Results were pooled using a random-effects model. Meta-regression determined factors associated with opioid use.

Results. Sixty studies (N=3,961,739) reported data on opioid use in people with chronic pain from 1990-2017. Forty-six (77%) had moderate risk of bias. Opioid use was reported by 26.8% (95%CI 23.1%-30.8%; moderate quality evidence) of people with chronic pain. The use of weak opioids (17.3% (95%CI 11.9%-24.4%; moderate quality evidence) was more common than strong opioids (9.8% (95%CI 6.8%-14.0%; low quality evidence). Meta-regression determined opioid use was associated with geographic region (P=0.02; lower in Europe than North America), but not sampling year (P=0.77), setting (P=0.06), diagnosis (P=0.34) or disclosure of funding (P=0.77).

Discussion. Our review summarised data from over 3.9 million people with chronic non-cancer pain reporting their opioid use. Between 1990 to 2017, one quarter of people with chronic non-cancer pain reported taking opioids and this proportion did not change over.

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Duration of postoperative opioid use after hip or knee surgery: a systematic review and meta-analysis

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Introduction: Major orthopaedic surgery such as hip or knee surgery is associated with severe pain and has the potential to lead to persistent postoperative opioid use, which contributes to the global opioid crisis.

Aims: To conduct systematic review and meta-analysis to identify the proportion of adult patients taking opioids at 3-12 months after hip or knee surgery. Secondary objective was to determine risk based on preoperative opioid use status.

Methods: A systematic literature review was conducted using EMBASE, MEDLINE, Cochrane Central Register of Controlled Trials and International Pharmaceutical Abstracts for articles published from 1st January 2009 to 24th June 2020. Only studies focusing on adults who underwent hip or knee surgery, with at least 3 months postoperative follow-up were included.

Results. In total 34 observational studies were included in the systematic review (n=865822). Of these, 16 reported hip surgery and 22 reported knee surgery. Six of them were conducted in veterans or military settings. In patients with hip surgery, postoperative opioid use was as follows: 3 months (21%, 95% CI [14%, 28%]), 6 months (18%, 95% CI [14%, 23%]), 9 months (22%, 95% CI [17%, 28%]) and 12 months (28%, 95% CI [26%, 29%]). In patients with knee surgery, postoperative opioid use was as follows: 3 months (23%, 95% CI [15%, 31%]), 6 months (20%, 95% CI [16%, 24%]), 9 months (5%, 95% CI [5%, 30%]) and 12 months (17%, 95% CI [4%, 31%]). Preoperative opioid users had higher opioid consumption at 3 months in patients with hip surgery (45% versus 4%) and knee surgery (55% versus 10%). Studies that were conducted in veteran or military setting reported higher proportion of postoperative opioid use compared to studies that were conducted in general population, especially for preoperative opioid user (higher than 50%).

Discussion: In patients who have hip or knee surgery, over 20% have persistent opioids use for longer than 3 months postoperatively and this may be sustained for over 12 months. Opioid naïve patients are less like to have continued postoperative opioid use compared to those who are opioid tolerant preoperatively. Clinicians involved in the care of these patients should be aware of this trajectory of opioid consumption after surgery and focus on deprescribing.

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Effect of *CYP2B6* 516G>T on Plasma Efavirenz and 8OH-Efavirenz Concentrations in Papua New Guinea HIV patients

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Introduction. Papua New Guinea (PNG) has the highest prevalence of HIV/AIDS in the Pacific with efavirenz (EFV) as the main treatment. EFV is mainly metabolised by CYP2B6 to 8-hydroxy-efavirenz (8OH-EFV). Very little is known about PNG EFV *CYP2B6* genetics and its relationship to plasma efavirenz and 8OH-EFV concentrations and of the metabolic ratio of 8OH-EFV to EFV.

Aims. To determine the frequency of *CYP2B6 516G>T* in PNG HIV/AIDS patients receiving efavirenz treatment and to examine the relationship of *516G>T* on plasma EFV, 8OH-EFV and the metabolic ratio.

Methods. Whole blood and plasma were collected from 154 PNG HIV/AIDS patients. EFV and 8OH-EFV plasma concentrations were determined by LCMS/MS. DNA was genotyped by MassArray panel through AGRF. Allele frequencies were compared to plasma EFV, 8OH-EFV and 8OH-EFV/EFV metabolic ratio. Further comparison was made between the genotypes and EFV therapeutic range (1-4 µg/mL).

Results. The T allele frequency was 53%. Of the patients that were homozygous variant (TT) for *516G>T*, 66% were above EFV therapeutic range and only 17% fell within therapeutic range. Metabolic ratio's range from 0.01 to 1.25 with an mean of 0.19.

Discussion. PNG HIV/AIDS patients exhibit very high frequencies of *CYP2B6 516G>T* variant genotype, TT. This genotype has shown to have an influence whether a patient falls within EFV therapeutic range in this population. These genetics may have important implications for CYP2B6 substrate drugs in this population.

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Current pharmacological and non-pharmacological interventions for illicit drug-induced presentations in emergency departments: A literature review

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Introduction. High prevalence of illicit drug use in Australia is a concerning issue. Patients repeatedly presenting to emergency departments (EDs) due to illicit drug-induced psychosis may contribute to overburdening of EDs.

Aims. To investigate the current pharmacological and non-pharmacological interventions provided in EDs.

Methods. Literature searches were conducted in the following databases: Ovid MEDLINE, PubMed, Embase Classic + Embase, Ovid Emcare and APA PsycInfo. Only English language manuscripts published between 2015 to 2020 were included. 58 manuscripts were identified, and 18 were included in this literature review.

Results. Cannabis and meth/amphetamine were common illicit drugs consumed by patients presenting at EDs with clinical presentations, such as agitation, aggression, and acute psychosis. Lorazepam, haloperidol and olanzapine were the most prescribed pharmacotherapies. Other medications included antidepressants, mood stabilisers, anticonvulsants, vasopressors, sodium bicarbonate, ketamine, propofol, antihistamines and antidotes, such as naloxone. Physical and mechanical restraints were the most common non-pharmacological interventions, followed by intravenous fluid replacement, intubation and ventilatory support, seclusion, bedside consultation, counselling, psychoeducation, cognitive behavioural therapy, and cognitive remediation therapy. However, some patients with self-limited symptoms were not given any medical treatment, despite demanding clinician attention.

Discussion. The pharmacotherapies reported in recent studies were in line with guidelines. While verbal de-escalation was the recommended first line treatment, it was not reported in any studies reviewed. Additionally, the use of restrictive interventions in these studies may be inappropriate. Patients with self-limited symptoms unnecessarily consumed ED resources contributing to ED overcrowding. Overcrowding and extended waiting time in EDs were identified as factors that precipitate violent and aggressive behaviours, posing significant risks to the safety of ED staff. Individuals with illicit drug-induced presentations were also more likely to re- present to EDs, even on multiple occasions, highlighting the significant burden illicit drug use places on ED resources.

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Development and validation of explicit criteria for identification of potentially inappropriate prescribing for people with type 2 diabetes mellitus

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Introduction. Early detection and timely resolution of potentially inappropriate prescribing (PIP) prevents adverse outcomes and improves patient care. There are many tools to identify PIP that target older populations, but an explicit tool specifically designed to detect PIP among people with Type 2 Diabetes Mellitus (T2DM) is lacking.

Aims. This study aims to develop and validate the Inappropriate Medication Prescribing Assessment Criteria to Type 2 Diabetes Mellitus (IMPACT2DM); an explicit tool that can be used to identify PIP for people with T2DM.

Methods. Updated national and international guidelines for the management of T2DM and drug information software programs were used to generate potential items. The content of the IMPACT2DM was validated by 2 consecutive rounds of Delphi method. Physicians and clinical pharmacists experienced in the care of diabetic patients and authors of selected diabetes guidelines were invited to participate in the Delphi panel. Consensus was assumed if 90% (first round) and 85% (second round) of expert panelists showed agreement to include or exclude an item.

Results. A total of 95 potential items were generated from selected diabetes guidelines and drug information software programs. In the first and second round there were 12 and 7 Delphi panellists, respectively. At the end of the first round 27 items had ≥90% agreement and were directly included in the final tool; 19 items were considered not PIP and were excluded from the tool. The second round contained 49 items; of these 43 were included and 6 were excluded. The final IMPACT2DM contains 70 items categorized based on the type of PIP and arranged in terms of medical conditions and medication classes.

Discussion. IMPACT2DM is the first explicit tool specifically designed to identify PIP for adults with T2DM. The tool can be applied using information on medical charts and requires minimal or no clinical knowledge. IMPACT2DM can be used by researchers and clinicians to assess quality of diabetes care, improve medication selection, and educate health professionals who are working with diabetic patients.

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Exploring the knowledge and attitudes of clients living with mental health conditions towards their medications and their healthcare providers.

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Introduction. It is vital that patients have an adequate level of understanding and knowledge of their medications for treatment success. However, medication education provided by healthcare providers may be inadequate. This is particularly concerning, especially for people living with mental health conditions as it has been previously reported that they receive less information from doctors and pharmacists than people with other medical conditions.

Aim. To explore the knowledge and attitudes of clients living with mental health conditions towards their medications and to study their experiences with healthcare providers.

Methods. Focus group sessions were conducted at a community-managed specialist mental health service provider with clients living with mental health conditions.

Results. Thirteen participants and two peer support workers participated in two focus group sessions. Responses indicated that participants did not feel confident in their knowledge of their prescribed medications as most participants did not seem prepared for the adverse drug reactions. This was commonly attributed to inadequate medication education, with a limited number reporting effective interaction with pharmacists. The majority of the participants also reported that they did not receive any form of written information and often resorted to seeking information on the internet. In addition, the participants expressed the need for a client-centred holistic care approach, involving direct communication between all healthcare providers.

Discussion. Findings indicated that there is a gap in medication education provided to clients living with mental health conditions, with the amount and quality of medication education given varying considerably. There is a need for immediate action to address this gap. Future research should consider exploring the scope for further medication education in the community setting such as at non-for-profit organisations.

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Medication safety during ICU care transfers with an Electronic Medication Management System

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9. Ana L. Hincapie, Winkle College of Pharmacy, University of Cincinnati, OH, USA

Introduction. For patients requiring admission to the Intensive Care Unit (ICU), transfers of care (TOC) during admission/discharge from the ICU are high-risk periods for medication errors. Within Australia, commonly general wards and the ICU do not share an integrated Electronic Medical Record and specifically an Electronic Medication Management System (EMMS) as part of the EMR.

Aims. To evaluate the effect of a hospital wide integrated EMMS on medication error rates during ICU TOC.

Methods. A 6-month historical control study was performed before and after implementation of the EMMS in the ICU of a tertiary hospital. Prescribing errors detected by pharmacists in the study period were divided into phase 1, (pre-EMMS, 6months), phase 2 (3 months post implementation after shakedown stage) and phase 3 (next 3 months of post implementation). They were categorized as prescribing error types under system or clinical intervention. Chi square statistics and interrupted time series analysis were used to assess the change in the proportion of patients who had an error at TOC during each phase. Logistics regression was used to determine the relationship between error type and study phase.

Results. TOC errors occurred in 42%, 64% and 19% of patients in phase 1, 2 and 3 respectively. There was a significant decline in the proportion of patients with an error between phase 1 and 3 ($p<0.01$). During phase 1, the proportion of patients with an error were increasing by 4.6 patients per month over the 6-months. Error rates reduced by 95% (95%CI= -103.5 to –46.7, $P<0.01$) by the end of phase 3. Of the error types, two system error categories 'wrong rate/frequency' and 'drug omission' showed a significant decrease between phase 1 and 3. All other error categories showed no significant change.

Discussion: Medication errors during TOC reduced following implementation of an integrated ICU EMMS. Added safeguards in hospitals such as, pharmacist interventions following implementation of an EMMS could reduce the risk of medication errors.

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Exploring Pharmacy Ethics in developing countries – a scoping review

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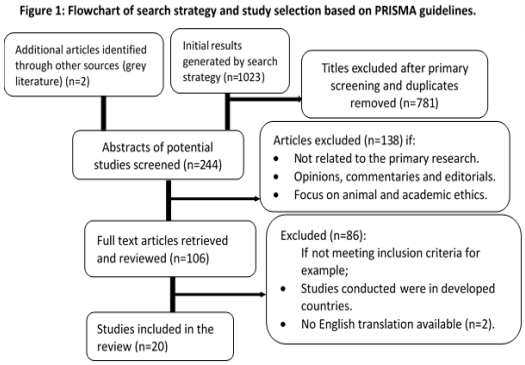
Introduction. Healthcare ethics have been profoundly influenced by principles of bioethics that emerged post-World War II in the Declaration of Geneva 1948. '*Beneficence*', '*Non-Maleficence*', '*Justice*' and '*Respect for Autonomy',* have become foundational principles of contemporary medical codes of ethics. These principles are well reflected in most professional pharmacy code of ethics globally. This domain remains relatively unexplored in most developing countries and the majority of what has been published in this area relates to Western cultures. There have been no attempts to pool findings from a similar scope of research emanating in developing countries.



Aim. This study aimed to explore the scope of pharmacy ethics in the literature pertaining to developing countries.

Methods. An extensive search of three relevant databases was conducted from Jan 2000 to July 2019, in order to identify relevant studies conducted in or focussed on ethics in pharmacy in developing countries.

Results. The full text of 20 relevant articles that met inclusion criteria were critically analysed and qualitatively categorised into three emerging themes; *Ethical challenges in pharmacy practice*, *Approaches used in teaching pharmacy ethics*, and *Code of ethics analysis and implementation.*

Discussion. Findings of this literature review illuminated a gap in pharmacy ethics literacy in developing countries and in pharmacists' ethical attitudes in handling ethical dilemmas, as well as a lack of familiarity with contemporary ethical principles and codes of ethics. In most developing countries pharmacists' lack of respect for patients' autonomy and pharmacists being prone to financial pressure were found to have a significant impact on pharmacy practice.

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Pharmacist's and physiotherapist's perspectives about sports pharmacy

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Introduction. Sports pharmacy is an emerging field. Although sports-related injuries in primary care are often managed by physiotherapists, consumers can seek advice and purchase medicines and products from community pharmacists. However, the scope of practice of pharmacists in sports medicine has not been reported.

Aims. This qualitative study aims to explore perceptions of pharmacists and physiotherapists about current and potential roles in sports pharmacy, to provide insight into barriers to, and facilitators of, pharmacist input.

Methods. A total sample (n=32) of equal numbers of pharmacists and physiotherapists was proposed. Using a snow-balling technique, AHPRA-registered pharmacists and physiotherapists were invited to participate in semi-structured interviews conducted from August 2020 (ongoing). Interviews were transcribed for qualitative coding and thematic analysis. Due to COVID-19, interviews were conducted via Zoom.

Results. Preliminary analyses from 11 interviews indicate that pharmacists currently have varied roles in what they perceive as being sports-related health care. Pharmacists are frequently called upon to provide sports-related health advice and are enthusiastic about their involvement. However, apparent barriers include lack of knowledge and training opportunities. Physiotherapists perceive the current role of pharmacists as being limited to the provision of medicines and medicines advice, and are willing to refer patients to a pharmacist for advice about the safe use of medicines. Physiotherapists are positive about collaborating with pharmacists in providing sports-related health care.

Discussion. Interviews and data collection are ongoing. Nevertheless, preliminary findings indicate that pharmacists are currently providing advice about a range of sports-related topics. Pharmacists feel most confident when providing advice about medicines, including prohibited substances, and triaging patients, but lack confidence in their knowledge about musculoskeletal injury and healing, supplements, strapping tape and devices/supports, despite receiving frequent requests for advice. Physiotherapists are positive about pharmacists' roles in sports health, but are uncertain about pharmacists' scope and expertise. Training opportunities and resources will be needed to support pharmacists in these roles, and future research should explore consumer perspectives about sports pharmacy.

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The psychosocial and work related impacts of the COVID-19 pandemic on Australian Pharmacists

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Introduction. The COVID-19 pandemic has led to unprecedented changes in the delivery of pharmacy services with pharmacists understanding they have an important role to play in the delivery of healthcare during this time. A changing work environment, and uncertainty are contributing to the psychological burden being felt by health professionals during the pandemic.

Aims. To determine the prevalence of burnout and the psychosocial and work related effects of the COVID-19 pandemic on Australian pharmacists.

Methods. A national survey was distributed to pharmacists throughout Australia using convenience sampling through social media and pharmacy professional organisations during April and June 2020. Burnout scores were calculated using the Maslach Burnout Inventory (MBI) and descriptive statistics were used to determine the effect of COVID on various work related and social variables.

Results. A total of 647 responses were received that contained full datasets to be analysed. Almost 40% of respondents were community pharmacists, 42.4% were hospital, 3.3% were from areas other than hospital/community pharmacy and 14.4% worked in a combination. The mean burnout scores for each of the burnout categories are presented in the table and indicate a higher degree of burnout than has been previously reported (Durham et al 2018). There were 35% of pharmacists that reported an increased workload during COVID however only 17.8% had directly cared for a COVID positive patient. Medicines supply issues, an increase in workload and patient incivility were rated as factors most likely to affect pharmacists at work. Pharmacists were somewhat concerned about their own health or the health of their families as a result of their work and 87.2% reported that COVID-19 had affected their personal life.

MBI category	Mean score	Standard deviation
Emotional exhaustion	28.5	13.39
Depersonalisation	7.98	5.64
Personal accomplishment	36.58	7.56

Discussion. The COVID -19 pandemic has had a profound effect on the work and lives of Australian pharmacists, with many pharmacists experiencing burnout during this time.

1. Durham ME et al (2018) Am. J. Health Syst. Pharm; 75:S93-100.

493

Deprescribing opioid analgesics: An overview of systematic reviews

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Introduction. Clinical practice guidelines suggest that opioid analgesics should only be prescribed when necessary, in the lowest effective dose, and for the shortest duration. Reduction of prescribed opioids can be challenging and interventions to assist deprescribing may be of value to health care professionals and opioid consumers.

Aims. To synthesise and evaluate published evidence from systematic reviews examining the effectiveness of interventions to support opioid deprescribing.

Methods. Comprehensive searches in CINAHL, Cochrane Library, EMBASE and MEDLINE were undertaken to identify systematic reviews which examined interventions for prescribed opioid reduction or cessation. Eligible articles were peer-reviewed systematic reviews, published in the English language from March 2010 to March 2020. The primary outcome was reductions in opioids, measured in morphine milligram equivalents. Secondary outcomes were assessed where reported and included pain scores, quality of life measures, adverse events, and physical and psychological function. Two reviewers independently extracted information and scored methodological quality using the Assessment of Multiple Systematic Reviews 2 (AMSTAR-2) tool. Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology was applied to assess the evidence quality, with evidence summarized and conclusions compared across reviews via narrative synthesis.

Results. Eighteen reviews were found eligible for inclusion. Pharmacological (n=3), physiological (n=10), psychological or behavioural (n=4) and health system interventions (n=4) were examined. Rates of opioid reduction and discontinuation varied widely across reviews and interventions. Pharmacological and psychological based interventions showed the greatest reductions in MME within the study periods. Similarly, evidence suggests some improvements in patient outcomes such as pain severity, physical and psychological function and quality of life when opioids are deprescribed.

Discussion. Several types of interventions may be effective in supporting opioid deprescribing. Positive clinical outcomes for pain severity and quality of life may result from efforts to deprescribe opioids.

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Extended roles in Aotearoa New Zealand community pharmacy: The views of service users

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Introduction. Internationally, pharmacy models of care, services and funding are developing to ensure better use of the skills of the pharmacist workforce. This trend is reflected in Aotearoa New Zealand (ANZ) with extended roles for community pharmacists (CPs) developing in relation to both individual patient care and population health. Role expansion has the potential to facilitate improvements in health outcomes and reduce inequalities.

Aims. To understand current developments in community pharmacy services in ANZ including the extent to which the expansion of roles is successfully occurring and what the enablers or barriers to progress might be.

Methods. Twenty-one semi-structured, audio-recorded interviews were conducted face-to-face or by telephone, with users of extended community pharmacy services. Participants were recruited in 2019/20 from 8 diverse case study sites. Interviews were transcribed verbatim, coded and analysed thematically.

Results. Preliminary analysis has identified that service users have mixed recognition of the different roles of staff within the pharmacy, with the role of pharmacy technicians poorly understood. A range of factors potentially influenced the uptake of extended services offered by the pharmacy including trust in the staff and a respectful and comfortable relationship, together with confidence in the CPs' knowledge and skills, along with ease of access to the pharmacy and convenience. Lack of awareness of the scope of extended services on offer was a potential barrier to uptake. Despite this, a positive experience with one specific service sometimes promoted an explicit acceptance of other roles and extended services. While the financial cost of a service was not necessarily a barrier for an individual interviewee, many acknowledged that it may be for others.

Discussion. A trusting and respectful relationship between pharmacy staff and service users, and optimal promotion of the extended services on offer are key facilitators to uptake. These case studies (also including interviews with a variety of pharmacy staff and local healthcare professionals) are the final stage of a larger study exploring the contexts in which changes in community pharmacy services in ANZ are occurring, the health and health service outcomes that are expected to result and the mechanisms producing change.

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Interventions at hospital discharge to guide caregivers in medication management for people living with dementia: A systematic review

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Introduction. Hospital discharge has a significant impact on the continuity of care for people living with dementia. Clear guidance on medication management should be provided to caregivers of people living with dementia to ensure appropriate use of medications post-discharge.

Aims. Identify and appraise the impact of interventions at hospital discharge to guide caregivers in the medication management for people living with dementia.

Methods. A systematic search of original studies was performed in Medline, Embase, PsycINFO and CINAHL. Articles published in English that reported on interventions to guide caregivers in medication management for people living with dementia were included. Two authors independently reviewed titles and abstracts.

Results. A total of five studies were included with a range of interventions that were typically delivered post-discharge by a multidisciplinary team and most targeted administration of medications by caregivers. Overall, three types of discharge interventions were identified including a pre-discharge caregiver educational intervention, a post-discharge caregiver support intervention, and medical discharge intervention at transitions of care. Of these, a pre-discharge caregiver education demonstrated shorter hospital stay (25 days vs 31 days, p=0.005). A post-discharge intervention that included follow-up visits reported lower use of high-risk medications (19% vs. 40%), and reduction in 30-day re-hospitalisation rates (11% vs 20%). In contrast, in another post-discharge intervention study, no difference in one-month re-hospitalisation rates (8.4% v 8.0%, p=0.818) was demonstrated. In another study, a post-discharge hospital educational program provided to caregivers found caregiver burden significantly decreased from 31.7 ± 17.6 (SD) pre-intervention to 27.7 ± 16.9 (SD) post-intervention (p=0.037).

Discussion. Current findings suggest there is a need for holistic interventions to guide caregivers in all aspects of medication management for people living with dementia, and should include support for caregivers in care coordination

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Role of medication beliefs on medication adherence in hypertensive Middle Eastern refugees and migrants in Australia

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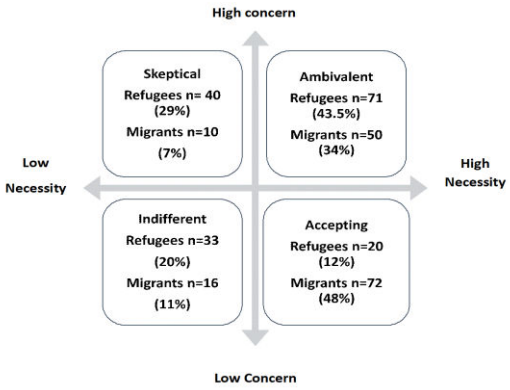
Introduction. Adherence to medications continues to rank as a major clinical problem in the management of patients with essential hypertension. Patients' behaviour of taking medications may be influenced by their beliefs about medications. Different populations such as refugees, and migrants may have different perceptions about their prescribed medications, which may influence their medication adherence.



Aims. To evaluate the impact of medication beliefs on medication adherence, and to assess the potential differences between refugees and migrants, in medication adherence and medications beliefs.

Methods. A cross-sectional study (n= 319 Middle Eastern refugees and migrants) was conducted using a survey that links Beliefs about Medicine Questionnaire (BMQ) and the Medication Adherence Questionnaire. BMQ scores (necessity and concerns scales) were classified as "accepting", "indifferent", "ambivalent" or "skeptical".

Results. There were significant associations between medication adherence and necessity and concerns scales. Refugees were likely to have less necessity, and more concern beliefs than migrants. They were also less likely to adhere to medications. Refugees and migrants with "accepting" beliefs reported the highest adherence to medication and those holding "skeptical" beliefs reported the lowest adherence.

Discussion. Following from the findings of this study, interventions to improve medication adherence need to focus on the 'skeptical' and 'ambivalent' clusters. Understanding the characteristics of each of these clusters by healthcare providers may lead to appropriate interventions for improving medication taking behaviours.

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A pilot pharmacist health coaching trial investigating changes to modifiable health behaviours

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Introduction. Pharmacists have used health coaching to improve patient management of hypertension in a number of international settings, but the provision of the service by Australian community pharmacists has been limited. During health coaching, the stages of change (SOC) approach can be applied to motivate and facilitate progress towards positive health behaviour change. This approach has been previously used by Australian pharmacists but has been confined to smoking cessation services. The application of the SOC model by pharmacists has involved interviews and questionnaires, which although convenient do not provide a realistic representation of the cyclic nature of the SOC. Thus, we have used a dynamic measure of SOC to evaluate the outcomes of health coaching by Australian community pharmacists in patients with poorly controlled hypertension.

Aims. To investigate whether pharmacist health coaching improves progression through the SOC for three-modifiable health behaviours: diet, exercise, and medication management in participants with poorly controlled hypertension.

Methods. Stages of change charts were developed for three-modifiable behaviours. In this pilot clinical controlled trial community pharmacist's health coached 20 participants with poorly controlled hypertension at monthly intervals. Changes in systolic hypertension and SOC with respect to the three modifiable health behaviours were assessed at session 1 and 4. To substantiate the behaviour change outcomes, SOC were also assessed in a validation group.

Results. Statistically significant changes in the modifiable health behaviours- medication management ($p = 0.03$) and exercise ($p = 0.01$) were apparent in participants who received health coaching and were evident through positive changes in the SOC charts. This correlated with a decrease in mean systolic blood pressure from session 1 to session 4 by 7.53mmHg ($p<0.05$). The participants in the validation group did not experience significant changes with respect to the SOC.

Discussion. Pharmacists successfully utilised the dynamic SOC tool to assess patient's readiness to change and facilitate progress in three modifiable health behaviours parallel to an improvement in systolic blood pressure. These results pave way for the application of the SOC tool by pharmacists to guide management of other chronic conditions.

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Exploring contributing factors of acute psychological impact in community pharmacists due to COVID-19 pandemic.

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Introduction. The world continues to suffer through the coronavirus pandemic. Community pharmacists working throughout the pandemic faced an 'onslaught' of frustration and confusion from the public whilst desperately trying to maintain access to required medicines for all Australians. The Pharmacists' Support Service (PSS) run by pharmacists for pharmacists, interns, and pharmacy students across Australia provides a call-in service.

Aim. To identify contributing factors of acute psychological impact from issues raised by pharmacists in their calls to the PSS during the pandemic.

Methods. De-identified data from all calls received by the PSS during the study period (February to May 2020) were categorised as those specifically mentioning COVID-19 and those that did not. Data from calls in the same period in 2019 served as a comparator. Categorical data were analysed using a two-tailed chi-squared test. Thematic analysis of the qualitative date was conducted.

Results. The PSS noted a 31% increase in calls during the peak of the pandemic when compared to the same period last year. Most callers were community pharmacists (average 91%) with 79% of all calls related to emotional or psychological issues, including stress, anxiety and concerns about their own mental health. Workplace conflicts and concerns over workload were other issues raised by pharmacists.

Discussion. A significant proportion of callers had discussed anxiety and stress, workplace conflict, perceived bullying, extreme workloads, and job dissatisfaction, highlighting the highly stressful work of community pharmacists during this pandemic. Existing literature from Australia and around the world has highlighted concerns about these increasing pressures and the impact on pharmacists and their ability to appropriately provide safe access to, and advice about medicines. To fully understand the long-term psychological impact of the current pandemic, a larger study following a cohort of community pharmacists longitudinally over the next few years is required.

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A qualitative study exploring barriers to and facilitators of medication adherence in Ethiopia: hospital pharmacists' perspectives

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Introduction. Ethiopian pharmacists are involved in providing direct patient care services that assist patients with medication adherence. However, no studies have explored pharmacists' perspective and experience of medication adherence.

Aims. To explore hospital pharmacists' insights into barriers to and facilitators of medication adherence in Ethiopia.

Methods. Semi-structured face to face interviews with hospital pharmacists, actively involved in direct patient care, were conducted via Zoom/Skype. All interviews were audiotaped, transcribed verbatim, translated into English and analysed using thematic analysis to identify main themes and subthemes.

Results. A total of 14, mostly male (12), participants participated in the study. Five main themes emerged including an overview of medication adherence and its assessment, perceived roles of pharmacists in medication adherence, enablers of, and barriers to medication adherence and ways forward. The majority of pharmacists perceived that challenges in medication adherence start with its assessment. This was thought to be due partly to lack of daily assessment in healthcare, absence of cost-effective and validated tools, and low use of combined tools in Ethiopia. Other barriers identified were dosage form preference, disease conditions, treatment, health care system, and the government/national policies at large. Availability of a counselling room and drug information centre, patients' interest and readiness for cooperation, subsidization and free drug programs, and supportive patient orientated pharmacy curriculum were some of the facilitators identified by pharmacists. Pharmacists suggested several ways forward specific to each identified barrier including prioritizing patients for available interventions and simplifying complex medication regimen and strengthening of the existing facilitators to improve medication adherence.

Discussion. The findings of this study could be integrated into intervention programs, policy and curriculum to improve medication adherence in daily clinical care, and research projects. Patients' preference for dosage forms has not been reported previously and should be considered along with medication complexity and medication knowledge when considering medication adherence.

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Implementation of a customised automation tool to optimise pharmacy practice

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Background. The implementation of the electronic Medical Record (eMR) has introduced many benefits to improving healthcare delivery. The literature cites conflicting reports with respect to the expected benefits in saving clinician time ; increased documentation and negative user experience.

Description. Two Queensland Health Hospital pharmacy departments implemented a customised automation tool, into their pharmacy workflow. It is a free, open-source scripting language for Windows that allows users to easily create small or complex scripts. It allows the creation of keyboard shortcuts which can automate repetitive tasks such as launching specific internet sites. Core activities in the pharmacy work practice were identified as being aligned to this technological process ; pharmacist medication annotations and electronic intervention forms.

Action. Using a prospective observational study design this project consisted of two parts; a four-week trial of the autotext tool, followed by a questionnaire to assess the perceived effectiveness. Prior to commencement of the trial, the autotext tool was built and customised to suit clinical pharmacist workflows. Education was tailored to staff at both sites on tool use.

Evaluation. In both hospitals over 70% of pharmacists either agreed or strongly agreed with all Likert statements which indicates that they believe the autotext tool will facilitate improved user experience, greater productivity, and improved workflow. The pharmacists at both sites found intervention documentation the most useful feature of the tool. At both sites over 90% of participants agreed that the autotext tool was easy to use. Education was identified as a key requirement.

Implications. The autotext tool is an effective method for improving workflow, reducing the time needed for documentation and increasing the time spent in clinical duties. The benefits of the tool were realised at both hospital sites.

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The impact of QHMAC LAM restrictions on the prescription qty of oxycodone prescribed on discharge

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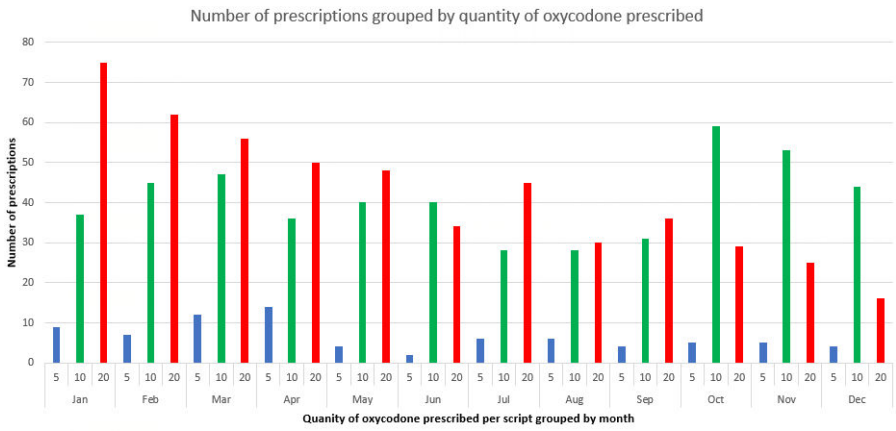
Background. Australia wide opioid supply has increased significantly over the past three decades with oxycodone being the main contributor. Several studies have shown that the quantity of opioids supplied on initial prescription positively correlates with the probability of prolonged unnecessary use. To address this, in July 2019 the Queensland Health Medicines Advisory Committee (QHMAC) made a formulary change which restricted the quantity of immediate release oxycodone 5mg tablets prescribed on discharge to a maximum of 10-tablets.



Aim. To determine whether the QHMAC formulary restrictions led to a reduction in the quantity of oxycodone tablets prescribed on discharge in a 300-bed outer metropolitan hospital.

Method. Data from the electronic prescribing software Cerner was downloaded for a 12-month period from March-2019 to February-2020 which captured 6 months of prescribing data pre and post the QHMAC intervention. The proportion of 20-tablet prescriptions was compared with the quantity of 5 or 10-tablet prescriptions.

Results. A Chi-squared analysis found a significant reduction in the proportion of 20-tablet prescriptions which coincided with an increase in the proportion of 5/10-tablet prescriptions in both the surgical (p=<0.001) and medical (p=0.001) divisions of the hospital. This suggests that the formulary change led the doctors to prescribe lower quantities of opioids on discharge. No change in prescribing practice was identified in the emergency department. A number of contributors to this result were considered; lack of pharmacist presence; emergent nature of presentations; rebound presentations out of primary care hours; prescriber education and support.

Conclusion. The results demonstrate the effectiveness of QHMAC formulary restrictions in driving practice change and reducing opioid prescribing. It is acknowledged that there are multifaceted factors of opioid use and prescribing but this single step should not be overlooked as a part of raft of interventions that can be used.

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An audit of the appropriateness of antipsychotic prescribing for cognitive impairment

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Introduction. The Aged Care Royal Commission highlighted the increasingly inappropriate use of antipsychotics for responsive behaviours associated with cognitive impairment. Despite limited published data on the extent that hospitals contribute to this problem, there are possible links between hospital prescribing and inappropriate continuation post-discharge.

Description. To assess the appropriateness of antipsychotic prescribing against industry guidelines in elderly patients over 65 years (over 45 for Aboriginal and Torres Strait Islander people) at a 300-bed regional Australian hospital.

Method. A retrospective audit of patients prescribed an antipsychotic between July and December 2019 was conducted to evaluate antipsychotic prescribing against the Australian Therapeutic Guidelines. Participants were excluded if they were receiving antipsychotic treatment for bipolar disorder, schizophrenia, post-operative nausea and vomiting or palliative care.

Results. The final cohort consisted of 141 participants, 36.9% being residential aged care facility (RACF) residents. First-line therapy including treatment of potential underlying causes of delirium and non-pharmacological interventions was documented in only 48.4% of patients. A new antipsychotic was prescribed in 75.9% of participants, 31.8% being RACF residents. Only 16.2% of all prescribed antipsychotic doses and frequencies aligned with industry guidelines, mostly due to regular dosing rather than recommended once-only or when-required dosing. While current guidelines advise review and immediate de-escalation of all antipsychotics upon symptom resolution, 48.2% of initiated antipsychotics were continued on discharge with just under 50% discharged to RACFs. Despite best practice guidelines recommending review of ongoing antipsychotic use, 47% of prescribed antipsychotics were unreviewed home medications and a mere 8.8% had documented plans for review post-discharge.

Discussion. This audit found antipsychotic prescribing had extremely poor adherence to industry guidelines, with an alarming number continued upon discharge without a documented plan for review. This potentially contributes to inappropriate antipsychotic use in RACF facilities and the wider community.

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Virtual Renovation of the Medication and Pharmacist Engagement Clinic (MAPEC)

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Background. In response to the Covid-19 pandemic, to ensure consistency with statewide directives of social distancing and where possible, to facilitate work from home models of care, the Medication and Pharmacist Engagement Clinic (MAPEC) at Ipswich Hospital underwent a virtual renovation.

Description. To continue the provision of a comprehensive medication counselling clinic, whilst minimizing face-to-face contact, MAPEC was overhauled to facilitate a virtual pharmacy clinic model. Instead of seeing patients in person, they were now seen via telehealth or phone calls by a pharmacist working from home.

Action. In addition to the use of standard remote desktop technology to access onsite programs such as ieMR and eLMS, two additional modes of technology were developed and incorporated to provide pharmacy care.

- The Queensland Health virtual clinic software – a program that facilitates face to face remote interaction via video calls. It was incorporated to enable personal and effective counselling.
- A customised data analytics application – developed in association with the IT department using the platform Qlik sense. The application is used to identify patients who had been discharged within the past 24 hours. In addition to pharmacist referrals, patients discharged out of pharmacy hours were identified and contacted, capturing a broader cohort than the prior model.

Evaluation. The virtual clinic model saw a 36% increase in the number of patients who were counselled from an average of 11 patients/day to 15 patients/day. The changes also enabled the pharmacists to contact patients discharged outside of business hours who would not usually receive a medication counselling service.

Implication. Developing and utilising technology in conjunction with a remote telehealth clinic can increase clinic capacity and enable follow up of patient cohorts who would ordinarily miss the pharmacy discharge service due to discharging out of hours. This can be achieved without a reduction in the quality of care provided.

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Evaluation of efavirenz and 8-OH-EFV exposure and the occurrence of drug-induced side effects in Papua New Guinea HIV/AIDS patients.

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Introduction. HIV/AIDS significantly impacts the health of the people of Papua New Guinea (PNG). Efavirenz (EFV) was prescribed as the preferred drug therapy for HIV/AIDS in PNG for two decades until recently, when it was replaced by dolutegravir. The unique genetics of PNG HIV/AIDS patients may place many of them at higher risk of major side effects related to high plasma EFV concentrations (Tucci et al., 2018). EFV is mainly metabolised to the inactive 8-OH-EFV.

Aims. Evaluation of EFV and major metabolite exposure through the quantification of plasma concentrations by LCMS/MS and side effects occurrence in PNG HIV/AIDS patients.

Methods. One hundred and fifty-five patients under therapy with EFV (600 mg/day) participated in the study after giving written informed consent. The plasma concentrations of EFV and 8-OH-EFV were determined by a LCMS8040 triple quadrupole MS using a C18 column for analytes' separation after supported liquid extraction. CNS and psychiatric side effects were evaluated with yes/no to symptom questions reported by the patients.

Results. Patient's age ranged from 14 to 65 years with body weights ranging from 36 to 120 kg. Mean±SD (range) concentrations for EFV and 8-OH-EFV were 2443±2303 (42 to 13211) and 271±205 (25 to 935) ng/mL, respectively. The mean 8-OH-EFV/EFV concentration ratio was 0.18±0.19 (0.01 to 1.25). Two patients had undetectable concentrations of EFV and 8-OH-EFV. Considering the EFV therapeutic range (1000-4000 ng/mL), 93 of the total patients (58%) had concentrations within, 36 (23%) below and 26 (16%) above the range. Eighty-five of 155 patients reported side effects, comprising 57 CNS, 8 psychiatric and 20 with both side effects. There was no apparent relation between plasm EFV or 8-OH-EFV and the occurrence of side effects.

Discussion. The results demonstrate extremely large interindividual variability in plasma EFV concentrations and metabolic ratios among PNG HIV/AIDS patients, likely due to CYP2B6 genetic polymorphism.

Tucci et al. (2018) Pharmacogenomics 28(6):153-164

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Cefepime-Induced Neurotoxicity: Identifying the Toxicity Threshold

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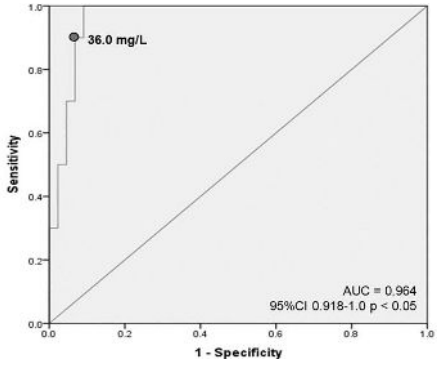

Introduction. Cefepime-induced neurotoxicity (CIN) has been demonstrated to be associated with cefepime plasma concentrations, however the toxicity threshold remains unclear.

Aims. The primary objective was to identify the cefepime plasma trough concentration at which neurotoxicity occurs. Secondary objectives were to determine the incidence of CIN at a tertiary institution, and to identify patient factors associated with the development of CIN.

Methods. A retrospective review of all patients administered cefepime between October 2017 and May 2018 in a tertiary hospital was conducted to determine total incidence of CIN. A Receiver-Operator Characteristic (ROC) curve was constructed to review the sensitivity and specificity of using various cefepime trough plasma concentrations to predict neurotoxicity. A regression was conducted to identify patient factors associated with CIN.

Results. In total, 206 patients were administered 259 courses of cefepime, with an overall incidence of CIN of 6% (16/259 courses). 64 courses had a cefepime trough concentration measured (24.7%). A cefepime trough concentration of 36mg/L provided the best differentiation between patients who experienced neurotoxicity and those who did not. No other patient covariates were identified to be significantly associated with CIN.

Discussion. A cefepime trough plasma concentration > 36mg/L was associated with CIN. The use of cefepime therapeutic drug monitoring may assist in the prevention of CIN by targeting trough concentrations < 36 mg/L. The retrospective design and small patient numbers were limitations of this study. A prospective study in a larger population is warranted given the wide disparity of results from existing studies investigating the toxicity threshold.

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Green tea extract and its metabolites induce biochemical changes linked to hepatotoxicity in HepG2 cells

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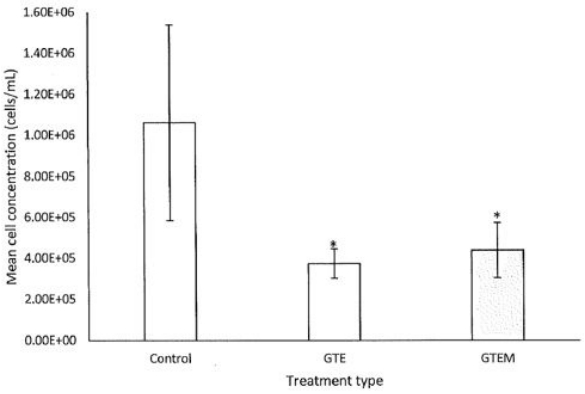
Introduction. Green tea extract (GTE) is commonly used for its wide range of purported health benefits, but has been implicated in over 50 cases of liver damage in the last 20 years (Mazzanti, 2015). However, little is currently known in regards to which biochemical pathways are affected during GTE-induced hepatotoxicity.



Aims. This study aimed to determine the chemical composition of GTE and its metabolites, and to examine the biochemical pathways that are affected by exposure of HepG2 cells to these compounds.

Methods. Chemical composition of GTE products was investigated using GC-MS. GTE and individual catechins were metabolised with S9 human liver fraction and profiled using GC-MS metabolomics. HepG2 cells were exposed to GTE, catechins and their metabolites for 24 h and resulting biochemical changes determined using GC-MS metabolomics.

Results. Metabolism of GTE occurred with 17 metabolites produced, 10 of which were also produced by metabolism of catechins. Exposure of HepG2 cells to GTE significantly decreased amino acids, oxoacids and carboxylic acids at 1 mg/mL, but produced a different profile at 0.1 mg/mL. Exposure to metabolites of GTE caused changes in amino acids, carbohydrates and fatty acids in all treatment groups.

Discussion. This study suggested that GTE causes disruption to cellular lipids, proteins, nucleic acids and the mitochondria in HepG2 cells. This corroborates existing data that GTE hepatotoxicity is a dose-dependent process that induces ROS production, ATP depletion and apoptosis. Regulation of herbal supplements containing this product must be improved to ensure consumer safety and prevent further cases of liver damage.

Mazzanti et al. (2015), Arch Toxicol. 2015;89(8):1175-91

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Exploring the TRAIL of doxorubicin-induced cardiotoxicity

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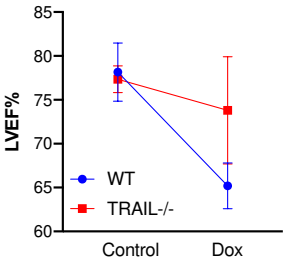
Introduction: Doxorubicin (DOX) is a widely prescribed chemotherapeutic used to treat both solid and haematologic malignancies. However, its use is limited by irreversible cardiotoxicity, which can lead to lifelong, sometimes fatal, heart complications. Recent evidence suggests the involvement of the TNF-related apoptosis-inducing ligand (TRAIL) which, through binding to its death receptors 4 and 5 initiates a signalling cascade leading to cell death. We hypothesise DOX upregulates death receptors in cardiomyocytes resulting in their sensitisation to TRAIL-induced death.



Aims: To investigate the role of TRAIL and its signalling pathway in DOX cardiotoxicity.

Method: Using cultured human cardiomyocytes, we assessed the ability of DOX to elicit cardiomyocyte death, and measured changes in death receptors using flow cytometry. Wildtype and TRAIL knockout mice (TRAIL-/-) (n=7 per group) were also used to evaluate the effect of TRAIL deficiency on cardiotoxicity following chronic DOX dosing. Cardiac function was assessed by measuring left ventricular ejection fraction (LVEF) and fractional shortening (FS) using echocardiography. T-tests and two-way ANOVAs were applied for statistical analysis where appropriate.

Results: In cell culture, we showed that (i) DOX treatment of cardiomyocytes was cytotoxic only in the presence of TRAIL; (ii) death receptor 5 on cardiomyocytes increased significantly (98%) with DOX treatment; and (iii) blockade of TRAIL signalling protected human cardiomyocytes from DOX-induced death. In wildtype mice, DOX caused a 15.6% ($p<0.0001$) and 24% ($p<0.0001$) reduction in LVEF (Figure) and FS respectively, whereas DOX treated TRAIL-/- mice had no significant reduction in cardiac function.

Discussion: Our data supports the hypothesis of DOX sensitisation of cardiomyocytes to TRAIL-induced death. Collectively, these findings strongly support TRAIL blockade as a novel therapeutic strategy to limit or eliminate DOX-induced cardiotoxicity and identify several targets for therapeutic intervention.

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Ascending Jacob's Ladder of Density Functional Approximations for *in silico* toxicological modelling

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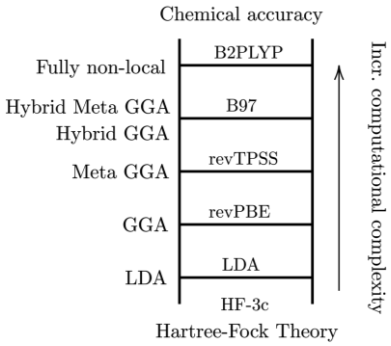
Introduction. The intricate balance between theoretical accuracy and computational cost is a current challenge associated with the use of density functional theory (DFT) methods in the development of quantitative structure activity relationship (QSAR) models. DFT is used to optimise 3D molecular structures and to precisely derive key reactivity descriptors from the molecular orbitals, which are highly applicable for modelling toxicological phenomena. Benchmark studies typically offer researchers guidance on their choice of density functionals by comparison of an often limited number of higher-level theoretical reference values [1].



However, the transferability of these theoretical evaluations to the predictivity and interpretability of resultant QSAR models remain unclear. Aims. This study will explore the predictive performance and interpretability of toxicological QSAR models generated using quantum mechanical (QM) descriptors computed at increasing levels of density functional theory.

Methods. Computation of the single point energies and 21 electronic descriptors of 8,755 chemicals from the Tox21 database [2] will be distributed across the Artemis high performance computing (HPC) cluster and these computations repeated at increasing levels of DFT from HF-3c at the LDA, revPBE, revTPSS, B97, and B2PLYP levels of theory. Multitask deep learning neural network models will be developed for each of the density functionals predicting 68 binary toxicological endpoints, optimised using genetic algorithms with cross- and external-validation, then interpreted using tree manifold and projection (TMAP).

Results. Preliminary results with HPC-distributed HF-3c calculations reduced total computation time from 78h to 3h compared to using a local desktop. Discussion. Improvements in the accuracy of toxicological QSAR models are expected at higher levels of DFT, however, we anticipate a compromise between accuracy gains and exponentially increasing computational costs to ensure these models can be practically utilised for regulatory purposes.

[1] Goerigk et al. (2017) Phys. Chem. Chem. Phys. 19: 32184. [2] Huang et al. (2016) Nat. Commun. 7: 10425.