P500. Computational video analysis for treatment and deprescribing evaluation in ageing mice

Kenji Fujita[1], John Mach[1] & Sarah N Hilmer[1]. Laboratory of Ageing and Pharmacology, Kolling Institute, Faculty of Medicine and Health,The University of Sydney and the Northern Sydney Local Health District, Sydney, NSW, Australia[1].

Introduction. In clinical observational studies of older adults, polypharmacy (use of ≥ 5 drugs) and Drug Burden Index (DBI; measures exposure to anticholinergic and sedative drugs) are associated with impaired physical function and frailty. Computational video analysis could be used as a tool to sensitively detect medication adverse effects.
Aims. Using computational video analysis of ageing male mice, we aim to examine the impact of medications on morphometric and gait function following chronic monotherapy, polypharmacy, and deprescribing (discontinuation).
Methods. Middle-aged (12 month) male C57BL/6 mice were chronically administered therapeutic doses of medications in polypharmacy regimens with different DBI (Zero DBI: simvastatin, metoprolol, omeprazole, paracetamol, irbesartan, Low DBI: simvastatin, metoprolol, omeprazole, paracetamol, citalopram, High DBI: simvastatin, metoprolol, oxybutynin, oxycodone, citalopram) or monotherapy with medications from the High DBI polypharmacy regimen. At age 21 months, half of the treated animals had the medications deprescribed. Open field videos (5 minutes each) were recorded and mouse clinical frailty index were assessed at 12, 15, 18, 21 and 24 months. After applying an open-source neural network to the videos, the gained features were analysed using a hierarchical Bayesian model that detects differences between the treatment groups and control over time.
Results. We measured 49 morphometric and gait features. Comparing all treatment groups to control, polypharmacy with High DBI induced the greatest number of differences at the earliest age and persisted to 24 months (Number of features altered at 15 months: Oxycodone:2, Oxybutynin:4, Citalopram: 16, Simvastatin:1, Metoprolol:2, Zero DBI:1, Low DBI: 17, High DBI: 28). Features that differed from control included decreases in distance travelled, rearing count, stride count, speed, body length, angular velocity, step length and increase in body width. Deprescribing reversed some outcomes. High and Low DBI polypharmacy and citalopram and oxybutynin monotherapy increased mouse clinical frailty index. For each treatment, different features were important in predicting frailty.
Discussion. Computational video analysis of preclinical open field data is a promising tool for automated, high-throughput, objective and sensitive detection of medication adverse effects. Polypharmacy with increasing DBI induced the greatest number of gait changes and frailty, and some effects were reversed with deprescribing.

P501. Sustainability of in-hospital deprescribing: a scoping review with a systematic approach.

Angela John[1,2], Nashwa Masnoon[2], Sarita Lo[2], Lisa Kouladjian O'Donnell[2], Edwin Tan[1], Sarah N Hilmer[2]. School of Pharmacy, University of Sydney[1], Sydney, NSW, Australia; Kolling Institute, University of Sydney and Northern Sydney Local Health District[2], Sydney, NSW, Australia.

Introduction: Deprescribing is a strategy to reduce medication-related harm, but little is known about the sustainability of hospital-initiated deprescribing interventions once patients are discharged.
Aims: To summarise literature on i) sustainability of in-hospital deprescribing changes (drugs remaining deprescribed) and ii) actioning of in-hospital deprescribing recommendations (deprescribing being implemented) after discharge. Methods: A scoping review with a systematic search was undertaken in MEDLINE from inception to March 31st, 2023 to include interventional studies with control groups (randomised and non-randomised) investigating sustainability of in-hospital deprescribing changes and actioning of in-hospital deprescribing recommendations. Inclusion criteria were inpatients with mean/median age ≥65 years, with follow-up of medications up to 12 months post-discharge.
Results: Five out of 338 studies met the inclusion criteria. All five were randomised controlled studies of deprescribing interventions. Mean/median age ranged from 76-80 years, number of medicines from 4.8-24 and comorbidities from 6-11. Two studies investigated sustainability of deprescribing, one study investigated actioning of recommendations, and two studies investigated both sustainability of deprescribing and actioning of recommendations. The change in the number of medicines from baseline (either discharge or admission) to follow-up, did not differ between intervention and control groups in two studies and there was significant reduction in the number of medicines in the intervention compared to control in three studies. One study investigated a more direct measure of recommendation implementation and reported that 80.1% of recommendations were fully or partially implemented after six months, but this included recommendations for starting and stopping medicines. All four studies reporting safety and efficacy outcomes such as hospitalisation and mortality found no significant differences between intervention and control.
Discussion: There are inconsistent findings regarding sustainability of in-hospital deprescribing interventions. Future studies using more direct measures, such as proportion of patients with deprescribing sustained or actioned and investigating specific patient, medication and disease-related factors impacting these findings may elucidate further insights into the outcomes of inpatient deprescribing interventions.

P502. Outcomes of considering goals of care in medication review: a systematic review

Nashwa Masnoon[1], Cristen George[2], Sarita Lo[1], Edwin Tan[2], Aagam Bordia[2], Sarah Hilmer[1]. Kolling Institute, Univ of Sydney and Northern Sydney Local Health District[1], Sydney, NSW, Australia; School of Pharmacy, Univ of Sydney[2], Sydney, NSW, Australia.

Introduction. Considering care goals in medication review may improve outcomes in older adults with polypharmacy.
Aims. To summarise literature on the outcomes of considering goals of care in medication reviews for older adults.
Methods. A systematic literature review was conducted by searching MEDLINE, EMBASE, SCOPUS and CINAHL databases from inception to March 2023, to identify studies that investigated patient-reported, prescribing and clinical outcomes of goal-directed medication reviews in study populations with mean/median age≥60 years.
Results. Seventeen out of 743 studies met inclusion criteria. Mean/median age ranged from 60.0-88.7 years, number of medicines from 7.7-17.7 and number of medical conditions from 3.0-16.9. In terms of patient reported goal achievement, 75% of patients achieved one or more goals in one study and 43% of goals were achieved in another study. Three studies compared patient-reported quality of life (QoL) between an intervention and control group. One study found significant improvement in QoL in the intervention group using the QoL for Osteoporosis questionnaire, one study found significant improvement using the EuroQol-Visual Analogue Scale (EQ-VAS) but no differences using the EuroQol-5 Dimension (EQ-5D) measure and one study did not specify if differences were significant as per quality-adjusted life years using EQ-VAS and EQ-5D. Two studies additionally used qualitative methods and found that patients perceived the intervention as a positive change which facilitates shared decision making. There were inconsistent findings regarding prescribing outcomes. For example, two studies compared the total number of medicines between intervention and control groups. One study reported this was significantly lower in the intervention group and another study reported no significant differences. All six studies comparing clinical outcomes such as falls and hospitalisation between intervention and control groups found no significant differences.
Discussion. Whilst there were inconsistent findings regarding prescribing outcomes and no significant differences in clinical outcomes, there is evidence of positive impact on patient-reported outcomes. Given frequent changes to clinical status in older adults which can result in ongoing changes to goals of care, future longitudinal studies may provide further insights on the ongoing impact of goal-directed medication reviews in older adults.

P503. Co-designing medication management resources for people with dementia and carers during hospitalisation

Alexander J Clough[1], Danijela Gnjidic[1], Elizabeth Manias[2], Carl Schneider[1], Yun-Hee Jeon[3], Natali Jokanovic[4], Jane Thompson[5], Timothy Chen[1], Mouna J Sawan[1]. Sydney Pharmacy School, Univ of Sydney[1], Camperdown, NSW, Australia; School of Nursing & Midwifery, Monash Univ[2], Clayton, VIC, Australia; School of Nursing & Midwifery, Univ of Sydney[3], Camperdown, NSW, Australia; Department of Infectious Diseases, Monash Univ[4], Melbourne, VIC, Australia; Member of the public[5], Canberra, ACT, Australia.

Introduction. People with dementia commonly experience medication-related issues during hospitalisation and at discharge, yet they and their carers receive little or poor medication management guidance at these transitions of care.
Aims. To develop and test new, co-designed, medication management resources provided to people with dementia and carers during hospitalisation and at discharge, with one for people with dementia and one for carers.
Methods. Resource content was informed by a previous literature search and qualitative research, with refinement from our expert advisory group involving people impacted by dementia. People with dementia, carers, and healthcare professionals were recruited for focus groups that were content analysed to explore participants' perceptions of the resources' topics, content, and layout, and how well they met their needs. The resources were refined by the research team based on feedback from the focus groups, a graphic designer modified the design, and final copies produced.
Results. Four focus groups were conducted, two with carers (n=4,3), and one each with people with dementia (n=3) and healthcare professionals (n=3). The resources provide information on six topics including: shared decision-making, medications that may affect cognition, and how to take part in hospital processes that involve. People with dementia indicated that the resource would be useful and liked the striking format of the resource, but suggested simplifying the language and reordering the content to begin with the discharge checklist. Carers similarly thought the resource would be valuable, but preferred to be referred to as "supporters" and wanted clearer information on further support. Overall, participants thought the resource would be most beneficial if provided at admission to refer to during the hospital stay.
Discussion. Using genuine co-design approaches, key content topics that support people with dementia and carers' involvement in medication management decisions were identified and used to develop novel resources providing this guidance. The resources will be user-tested and formatively evaluated in further studies before the feasibility testing.

P504. Development of a tool to evaluate the organisational culture of residential aged care facilities and psychotropic medicines use in residents: A cognitive interview study

Boyi Chen[1], Timothy Chen[1], Yun-Hee Jeon[2], Jane Thompson, Danijela Gnjidic[1], Mouna Sawan[1].
Sydney Pharmacy School, Faculty of Medicine and Health, Univ. of Sydney[1], Camperdown, NSW, Australia; Sydney Nursing School, Faculty of Medicine and Health, The Univ. of Sydney, Camperdown, NSW, Australia[2]

Introduction: The high use of psychotropic medications continues to be common in residential aged care facilities (RACFs) despite the risk of harm and limited efficacy in people with dementia. Research has shown that the organisational culture of RACFs influences psychotropic medication use. The PRACTICE (Psychotropic medicines use in Residents And Culture: Influencing Clinical Excellence) tool was developed by the research team to comprehensively evaluate the organisational culture of RACFs specific to the use of psychotropic medications.
Objectives: To evaluate the understandability and relevance of the items of the PRACTICE tool.
Method: RACF managers, nurses, care staff, geriatricians, general practitioners (GP), pharmacists were invited, by advertisement via professional contacts and StepUp for Dementia Research, to participate in on-on-one cognitive interviews. Approximately 20 cognitive interviews will be conducted (or until data saturation is reached). Interviews were performed using the combination of think aloud technique and verbal probing, transcribed, and content coded using NVIVO for participants' perceptions of the PRACTICE tool.
Results: To date, cognitive interviews have been conducted with eleven participants. Stakeholders taking part included one GP, two aged care pharmacists, one community pharmacist, two RACF general managers, one RACF care manager, two nurse practitioners, one registered nurse, and one personal care assistant. Most participants reported to understand the items in the PRACTICE tool and several items were suggested for rewording to improve their comprehensibility. All participants considered items in the PRACTICE tool is acceptable for the evaluation of organisational culture related to psychotropic use in RACF.
Conclusion: Our next phase of this work will involve validation assessment of the PRACTICE tool is needed so that it can provide feedback on the key aspects of culture that require improvement to reduce psychotropic medication use.

P505. Patient's experience of a psychiatric, alcohol and non-prescription drug assessment (PANDA) unit

Hannah G Paul[1], Yvonne Nguyen[2,3], Farzad Jazayeri[2,4], Paul T Presiz[2,4], Jacqueline Huber[2,3], Jonathan Brett[2,5], Jane E Carland[2,5] Faculty of Medicine and Health, UNSW Sydney[1], NSW, Australia; School of Clinical Medicine, Faculty of Medicine and Health, UNSW Sydney[2], NSW, Australia; Dept Psych, St Vincent's Hosp[3], Sydney, NSW, Australia; Dept Emergency Med, St Vincent's Hosp[4], Sydney, NSW, Australia; Dept Clin Pharmacol & Toxicol, St Vincent's Hosp[5], Sydney, NSW, Australia.

Introduction. Individuals with psychiatric disorders (PDs) and substance use disorders (SUDs) possess unique psychosocial and health factors which act as barriers to accessing care. To overcome some of these barriers St Vincent's Hospital Sydney implemented the psychiatric, alcohol and non-prescription drug assessment (PANDA) unit. PANDA is tailored to provide care across a short length of stay for patients with PDs and/or SUDs who present to the hospital's emergency department.
Aims. To describe the participants' experience and opinions of the PANDA unit.
Methods: A mixed-methods design was used: (1) interviews with PANDA patients (n=14) to capture the patient experience of the unit, (2) a retrospective audit of medical records to describe participant demographics and admission characteristics. De-identified transcripts were analysed using an inductive approach to generate themes. Results: Most participants presented to the PANDA unit with alcohol intoxication/overdose/withdrawal (n=10). Three themes capture the patient experience of PANDA; PANDA is a safe space (physical safety and treatment without stigma); PANDA provides patient-centred care in a busy environment through an efficient and skilled multidisciplinary team (patients receive care from multiple healthcare professionals and are involved in decision-making); 3) PANDA facilitates further care (healthcare professionals help patients engage with services within and beyond the unit).
Discussion: PANDA provided a safe environment for patients to recover while receiving a wide range of tailored care from an efficient multidisciplinary team. Skilled staff provided care without stigma and integrated care beyond the acute patient presentation. Discharge planning sought to overcome patient-level barriers that may reduce access to future care. Exploring patient uptake of recommendations on discharge could help identify remaining barriers to care access.

P506. Identifying priorities for medication management resources for people living with dementia and their carers through community action

Mouna Sawan[1], Amanda Cross[2], Natali Jokanovic[3], Karen Watson[4], Jacqeline Wesson[5], Danijela Gnjidic[1], Alexander Clough[1], Fleur Harrison[1].
Sydney Pharmacy School, Univ. of Sydney[1], Camperdown, NSW; Centre for Medicine Use and Safety, Monash Univ.[2], Parkville, Vic; The Alfred Hospital and Monash Univ[3], Melbourne, Vic; Sydney Nursing School, The Univ. of Sydney[4], NSW, Australia; School of Health Sciences, Univ. of Sydney[5], Camperdown, NSW.

Introduction. People with dementia and their carers are given limited guidance in medication management which contributes to medication-related harm. Importantly, there are no resources that provide comprehensive medication management guidance across care settings. To fulfill this area of need, and ensure that resources are genuinely co-designed, the priorities for medication management resources need to be identified from the community.
Aims. To identify community-centred priorities for medication management guidance resources for people with dementia and their carers.
Methods. We established a 23-member consortium partnership with people living with dementia, carers, healthcare professionals, and national consumer and professional organisations using a community-based participatory research approach. The partnership will map out priorities for medication management resources and establish consensus using findings from a literature search, focus groups, and a modified Delphi survey. Focus groups commenced on the 25th of August 2023 and are currently ongoing. Qualitative data was content analysed to generate a list of priorities.
Results. To date, we have conducted three focus groups with the partnership. Preliminary analysis indicates medication management for changed behaviours, information on the rights of the person in shared decision making and clear guidance on assistance with medication administration are to be included among the generated list of community-centred priorities for medication management resources for people with dementia and their carers.
Discussion. This is the first time a community action approach has been adopted to co-design resources to support people with dementia and carers in medication management challenges. The community-centred priorities will be used to generate an inventory of consumer-tailored communication strategies for people with dementia and their carers.

P507. The effect of alcohol consumption on blood glucose in young adults with type 1 diabetes mellitus

Amy (Yoonseo) Chung[1], Benjamin J Wheeler[2], Hesham S Al-Sallami[1]. Otago Pharmacometrics Group, University of Otago[1], Dunedin, Otago, New Zealand; Women's and Children's Health, Dunedin School of Medicine[2], Dunedin, Otago, New Zealand.

Introduction. Precise glycaemic control is required to prevent debilitating short and long-term complications in Type 1 diabetes (T1DM). Ethanol complicates glycaemic control by inhibiting gluconeogenesis and glycogenolysis. The extent and time-course of this inhibition varies and can result in potentially life-threatening hypoglycaemia several hours after alcohol consumption.
Aims. To determine how alcohol affects blood glucose in patients with T1DM, and how other variables, such as physical activity and bolus insulin dosing, influence glycaemic control in these patients.
Methods. This was a prospective, observational study of young adults with T1DM. Participants provided 10-day worth of data of blood glucose, insulin dose and dosing time, food intake, physical activity, alcohol intake, and blood alcohol. Data were summarised and used to determine glycaemic control, incidences of hypoglycaemia, and variables associated with hypoglycaemia.
Results. Preliminary results (n=5) showed significant between-subject variability in glycaemic control. One participant had a spike in their blood glucose (18.9 mmol/L) at the same time as a blood alcohol concentration of 0.054% w/v. Low blood glucose was reported but no incidences of moderate or severe hypoglycaemia. Another participant measured 9.2 mmol/L in blood glucose at 0.022% blood alcohol concentration and quickly peaked at 22.1 mmol/L two hours later. No episodes of hypoglycaemia were detected.
Discussion. Data showed suboptimal glycaemic control associated with events when alcohol is consumed. A likely reason for this is the consumption of high-sugar pre-mixed drinks and eating sugary and carbohydrate-rich snacks. It is also possible that participants would reduce or omit their insulin dose in order to avoid severe hypoglycaemia later on.

P508. Cannabidiol in sport and exercise

Michael Kennedy. Clinical Pharmacology and Toxicology St Vincent's Hospital UNSW, Sydney, NSW, Australia.

There had not been any scientific investigation of the place cannabidiol (CBD) in sport or exercise prior to the 2017 decision by World Antidoping Agency (WADA) to allow participants to use CBD while continuing to prohibit use of tetrahydrocannabinol (THC). Suggested indications for its use in sport relate to anti-inflammatory, analgesic, anxiolytic, neuroprotective, sleep improvement and recovery enhancement properties. Major sporting organisations, events and eminent sportspeople now promote its use as an adjunct to sporting activity. Since 2017 there have been seven published studies and one Masters thesis in the refereed literature. These comprise 137 male and female subjects with varying levels of fitness from "healthy" to elite athletes. Double blind comparisons were made between oral or sublingual formulations of CBD in doses ranging from 16.67mg sl to 408 mg po to varying formulations of placebos or no intervention. Parameters evaluated included measures of aerobic performance ,strength, muscle soreness , muscle damage, markers of inflammation, health and well-being, and concentrations of CBD and anandamide. When compared to placebo CBD showed a slight decrease in muscle soreness in one, a small increase VO_2 max in one, deceases and minor deceases in biochemical measures in three. The longest study lasted eight weeks and did not show alterations in strength, VO_2 max or psychological parameters compared to control measures with CBD but strength decreased in the placebo arm. Conclusion: At present there does not appear be any established indication for the use of CBD in sport or exercise.

P509. Routine Therapeutic Drug Monitoring of Rivaroxaban: Experience at a Tertiary Center

Paul KL Chin[1,2], Adele O'Mahoney[2], Isabel Hiskett[2]. University Of Otago Christchurch[1], Canterbury, New Zealand. Te Whatu Ora Health New Zealand – Waitaha Canterbury, New Zealand.

Introduction. A liquid chromatography-mass spectrometry assay to determine plasma rivaroxaban concentrations has been available for routine clinical use at our tertiary hospital centre since 2017.
Aims. To describe (1) the use of the assay during July 2021 to March 2023; (2) the indications for testing; and (3) subsequent rivaroxaban prescribing decisions.
Methods. Patients for whom rivaroxaban concentrations were measured were identified using the laboratory database, and clinical data were extracted from the associated electronic health records.
Results. During the 22 months, there were 187 unique patients (female 54%) with median (range) age 75 (24-96) years that had 235 samples. The majority were anticoagulated for atrial fibrillation and treatment of venous thromboembolism, comprising 51% and 44%, respectively. The use of the rivaroxaban assay increased overtime, with a mean (95% CI) increase of +0.4 (0.1-0.7) samples per month. The median (range) rivaroxaban concentration was 78 (6-810) microg/L. The main reasons for testing were bleeding/thromboembolic event with rivaroxaban (22%), post-rivaroxaban initiation (22%), repeat sample post-rivaroxaban dose adjustment (11%), uncertainty about impact on renal function and drug-drug interactions (9%), concerning coagulation test result (7%). After the assay result, rivaroxaban dosing was decreased in 7% (17/235), increased in 3% (8/235), discontinued in 6% (15/235), continued in 79% (183/235) and unknown in 5% (12/235).
Discussion. The clinical use of the rivaroxaban assay has increased, with 17% of results associated with a subsequent change in rivaroxaban prescribing.

P510. An efficient HILIC-MS/MS method for monitoring metformin in human plasma

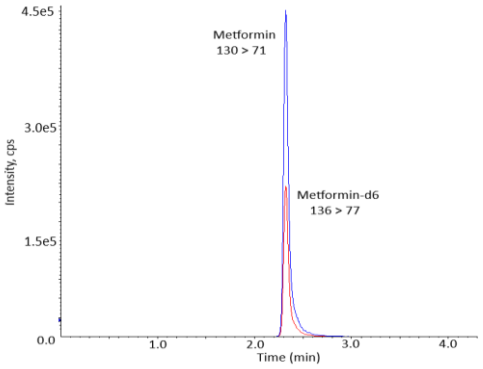
Mei Zhang [1,2], Grant Moore [2], Paul KL Chin[1], Matthew Doogue[1], Department of Medicine, University of Otago - Christchurch [1]; Toxicology, Canterbury Health Laboratories [2], Christchurch, New Zealand

Introduction. Concentration monitoring of metformin is considered necessary to reduce the risk of adverse effects for patients with severe renal impairment. The liquid chromatography/tandem mass spectrometry (LC-MS/MS) is the gold standard method for drug monitoring in biological samples. Metformin is highly polar which makes it difficult to extract from biological samples and to retain on stationary phases when using reversed phase chromatographic separation. Hydrophilic interaction liquid chromatography (HILIC) has been demonstrated to be a powerful technique for the analysis of polar compounds.

Aims. To establish and validate a rapid, simple and sensitive HILIC-MS/MS method for monitoring metformin in human plasma.

Methods. A simple one-step protein precipitation was used for plasma sample clean-up. Metformin and the internal standard metformin-*d*6 were resolved on a Kinetex HILIC column using gradient elution of 10 mM ammonium acetate containing 0.05% formic acid and acetonitrile, and then were analyzed by performing multiple-reaction monitoring (MRM) scans in positive electrospray ionization mode.

Results and Discussion. The total analysis time was 6.0 min. Standard curve was adequately fitted by quadratic equations (r > 0.999) over the metformin concentration range of 0.005 to 10 mg/L. No significant matrix effects were observed. The accuracy and precision of the assay were acceptable according to the U.S. Food and Drug Administration (FDA) regulations for the validation of bioanalytical methods. The assay has been used successfully in clinical practice to enhance the safe and effective use of metformin. An example of the clinical use of the assay can be seen in the figure.



Chromatogram of serum sample from a patient on metformin (Metformin = 5.74 mg/L)

P511. Equity and Quality Use of Medicines in people presenting to PANDA Unit

Elysse B Choy[1,2], Bridin P Murnion[2,3], Jana Stojanova[2,3]. School of Biomedical Sciences, UNSW Sydney[1], NSW, Australia; Dept of Clinical Pharmacol and Toxicol, St Vincent's Hosp Sydney[2], NSW, Australia; School of Clinical Medicine, UNSW Sydney,[3] NSW, Australia.

Introduction. The Psychiatric Alcohol and Non-Prescription Drug Assessment (PANDA) Unit at St Vincent's Hospital Sydney is an acute-care ward for patients experiencing drug and alcohol or mental health crisis. Despite policies and frameworks supporting Quality Use of Medicines (QUM), PANDA patients are at high risk of facing health inequity.

Aim. To assess QUM in PANDA patients using routine medication prescribing and administration data.

Methods. We conducted a retrospective review of electronic medical records including medication charts and digitised discharge summaries for all patients admitted in the period 11/20-12/21. We evaluated national QUM Indicators and Prescribing Safety Indicators relevant to this population. Indicators requiring discharge summary review used a randomly selected subset of patients. Polypharmacy was evaluated using a polypharmacy risk assessment tool.

Results. 983 episodes of care representing 651 patients were evaluated for polypharmacy risk. Most episodes involved a medium or high risk of polypharmacy (n=790/983, 80.36%). Co-prescription of antipsychotics and QT-prolonging drugs was common, and most patients with this prescription had this combination administered (n=119/136, 87.50%). Medication orders for 'as required' psychotropics mostly included an indication (n=170/253, 67.19%). Antipsychotic polypharmacy at discharge was low in patients prescribed antipsychotics during admission (n=2/103, 1.94%). Best possible medication history was documented in a minority of patients (n=42/103, 40.78%), and discharge summary availability was low for patients receiving discharge medications (n=33/61, 54.10%).

Discussion. Polypharmacy and use of high-risk medicines in PANDA are common due to the patient profile, including behavioural disturbance and substance withdrawal/intoxication requiring sedation and alcohol withdrawal protocols. These protocols also lead to the potentially harmful co-prescription of antipsychotics and QT-prolonging drugs. While electronic prescribing systems/medical records are useful to ensure medication order clarity, there is scope for improvement in communication at interfaces of care, including for medication reconciliation on admission and discharge planning to address the prevalent medication management challenges in the vulnerable PANDA cohort.

P512. Ethnic differences in the safety and efficacy of tyrosine kinase inhibitors

Nicki M. Kyriacou[1], Annette S. Gross[1], Andrew J. McLachlan[1]. Sydney Pharmacy School, Faculty of Medicine and Health, University of Sydney[1], Sydney, NSW, Australia.

Introduction. Population differences in intrinsic and extrinsic factors can influence the pharmacokinetics (PK) and pharmacodynamics (PD) of tyrosine kinase inhibitors (TKIs), resulting in variations in TKI drug response in populations of different ethnicities and geographic ancestries (Touma et al, 2017).
Aims. To identify and explore reports of ethnic differences in the efficacy and safety of TKIs used in the treatment of solid tumours and haematological cancers.
Methods. A literature search of Phase 3 clinical trials of 53 oncology TKIs was performed to collate population subgroup analyses of hazard ratios (HR) of two efficacy endpoints: progression free survival (PFS) and overall survival (OS). The PFS and OS data were represented by participant race/ethnicity/ancestry/geographic-region using forest plots (*forestplot* in *R*). For TKIs with suggestions of PFS and OS variation across populations, differences in the safety and intrinsic/extrinsic characteristics relevant for drug PK and PD were explored for each population.
Results. Results from 23,326 trial participants enrolled in 40 Phase 3 clinical trials (26 TKIs) for the treatment of 14 different cancers were evaluated. In most studies (n = 31), the efficacy outcomes of TKIs did not differ significantly across different races/ethnicities/ancestries/geographic-regions. Nevertheless, 9 trials indicated differences across subpopulations. For example, use of pazopanib as a maintenance therapy for ovarian cancer showed a reduced PFS benefit in East Asians (PFS HR: 1.16) relative to the White population (PFS HR: 0.69) (Du Bois et al, 2014).
Discussion. The grouping together of results from ethnically diverse participants, underrepresentation of specific populations and inconsistencies in the definitions of participant ethnicity/ancestry do not allow for meaningful comparisons and assessments of potential inter-ethnic/ancestry differences. Further research exploring the influence of ethnicity/ancestry determinants of TKI response is warranted to understand the contribution of these factors and to enable individualisation of drug selection/dose to optimise therapeutic outcomes in all patients.
Du Bois A et al (2014) J Clin Oncol 32:3374-3382
Touma JA et al (2017) Transl Cancer Res 6:S1558-S1591
Funding: Peter Coates Postgraduate Scholarship in Ethnopharmacology

P513. Four-year review of New Zealand laboratory infliximab and adalimumab concentration results indicating high potential for improved dosing

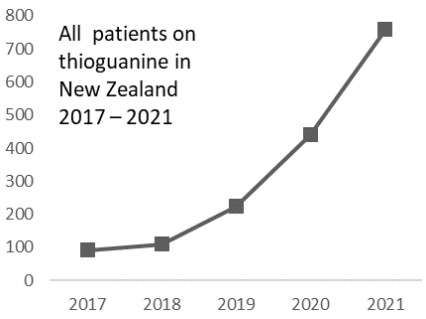
Murray L Barclay[1,4], Paula E Keating[2], Barry D Hock[3], Stewart M Smith[2], Paul KL Chin[1,4], John L O'Donnell[2]. Dept of Clinical Pharmacology[1], Christchurch Hospital, Dept of Immunology[2], Canterbury Health Laboratories, Waitaha Canterbury, Te Whatu Ora, & Depts of Haematology[3] and Medicine[4], University of Otago, Christchurch, New Zealand.

Background: Therapeutic drug monitoring (TDM) of infliximab and adalimumab, for inflammatory bowel disease (IBD) and rheumatology conditions, is routine in many countries and is recommended in national and international guidelines. Since 2018, a TDM service has been provided at a single laboratory in New Zealand with drug concentrations above 7mg/L being considered therapeutic.
Aims: A review of all test results for infliximab and adalimumab concentrations and anti-drug antibodies (ADA) at Canterbury Health Laboratories (CHL) between Feb 2018 and Jan 2022 was completed.
Method: Drug concentration was measured by ELISA/competitive homogenous mobility shift assay (HMSA). When the drug concentration was <2mg/L, samples were reflex tested for ADA by a HMSA method.
Results: Test numbers grew steadily over the 4 years, 2757 in 2022 with a cumulative total of 6591 (3782 infliximab and 2809 adalimumab). The total number of individual subjects was 2551. The median serum concentration for infliximab was 5.7 mg/L and for adalimumab 5.5 mg/L. Subtherapeutic drug concentrations (<7mg/L) were measured in 54% of samples. Drug concentrations <2mg/L were measured in 23% of samples with ADA detected in 51% of these. The ADA positive samples (12% of total samples received) were evenly split between weak positive, positive or strong positive.
Discussion: The high number of samples with subtherapeutic drug concentrations and common ADA detection is consistent with failing therapy but could also suggest that standard dosing is too low on average. These results reinforce the value of anti-TNF drug TDM in making decisions to adjust dosing or switch agents in patients on infliximab and adalimumab.

P514. Thioguanine efficacy, safety and TDM in IBD patients in New Zealand

Murray L Barclay[1-3], Crispin Ovenden[1], Ashley Anderson[1], Eunice Chou[1]. Depts of Clinical Pharmacology[1] & Gastroenterology[2] Christchurch Hospital, Te Whatu Ora – Waitaha/Canterbury and Dept of Medicine[3], University of Otago Christchurch – Te Whare Wānanga Otāgo ki Ōtautahi, New Zealand.

Introduction: Thioguanine (TG) has shown good efficacy and acceptable safety in patients with IBD in large studies from the Netherlands and the UK. There are now >1,000 patients on TG in NZ.
Aims: To replicate the Dutch and UK findings in a cohort of NZ patients.
Methods: A retrospective single centre study was undertaken. Patients prescribed TG for IBD under the care of a gastroenterologist in Canterbury were identified cross-checking a laboratory database of thiopurine methyltransferase activity results and thiopurine metabolite concentrations with pharmacy dispensing data. Electronic health records were searched for data on efficacy and adverse reactions. Efficacy was determined by persistence on therapy without steroids, surgery, or escalation to a biologic.



Results: Of 143 patients identified between 2016 and 2022, 63% had Crohn's disease and 35% UC. 141 had previously failed conventional thiopurines. Median daily TG dose was 20mg/day (range 2.9-40). Eighty percent of patients remained on TG at 12 months. Of those prescribed TG as concomitant immunosuppression while on a biologic, 50% were maintained without steroids or surgery. Of those not on biologic therapy, 38% persisted on TG without escalation to steroids, a biologic or surgery. Adverse effects were noted in 23% of patients, mostly mild or moderate, and many transient. One patient had pancytopenia from TG and recovered. The median measured 6-thioguanine nucleotide concentration was 724 pmol/8x10^8RBC (range <30 - 2538).
Discussion: Almost all patients in the cohort previously failed conventional thiopurine but 80% of patients remained on TG at 12 months. TG had good efficacy and low toxicity, with only one serious adverse event of pancytopenia and no nodular regenerative hyperplasia. This experience is now leading to TG becoming the first line thiopurine in many IBD cases in New Zealand.

P515. The Utility of Education Materials and Decision Aids to Guide Medication Management: A Scoping Review

Joseph Boutros[1], Carl R Schneider[1], Danijela Gnjidic[1]. School of Pharmacy, The University of Sydney[1], Sydney, NSW, Australia

Introduction. Medication management is an essential component of healthcare involving collaboration between patients and healthcare professionals to improve the safety and effectiveness of treatments. Education tools and decision aids are established approaches to facilitate shared decision making for medication management. An emerging interest has been to broaden the usability of these resources by culturally adapting them to diverse populations.
Aims. To explore the availability of culturally adapted education tools and decision aids in medication management and assess their utility.
Methods. A search strategy on five databases (EMBASE, MEDLINE, CINAHL, PsycINFO and Cochrane) was conducted alongside Google Scholar, to identify any primary sources or grey literature available in this field (to April 2023). Results were categorized based on Steinman and colleagues' description of 'The Enhanced Monitoring Framework': drug initiation, education, monitoring and follow-up (change, reduce, add, stop or maintain).
Results. Eighteen records satisfied the inclusion criteria from 928 potential records. Sixteen reported on education materials, and two on decision aids. The decision to initiate a therapy was the objective of only one tool (n=1), relating to smoking cessation. As such, no tools provided guidance on symptom burden and referral criteria. Most resources addressed education on symptoms, adverse events, disease states and inhaler technique (n=15), followed by monitoring and adherence (n=5). Follow-up was addressed in one resource (n=1). A range of cultural adaptation and validation methods were utilized, with language translation (n=17), assessment of the new cultural context (n=10) and internal consistency measurements (n=8) most common. Tools assessing patient knowledge produced varied results on medication management outcomes, particularly increased adherence (n=3).
Discussion. Among the number of culturally adapted resources in medication management that have been recently developed, the majority have been used to educate on medication monitoring. However, there is a need to expand and assess the applicability of these tools to more populations and focus on other medication management steps, especially the initiation and deprescribing of therapy.

P516. The effect of compulsory indications in electronic hospital prescriptions on prescriber behaviour

Lorna Pairman[1], Paul Chin[1,2], Richard McNeill[2], Matthew Doogue[1,2]. Department of Medicine, University of Otago[1], Christchurch, New Zealand; Department of Clinical Pharmacology, Te Whatu Ora Health New Zealand – Waitaha Canterbury[2], Christchurch, New Zealand.

Introduction. Recording the indication for a medicine in the prescription supports communication and reduces errors. In electronic prescriptions the indication field can be made compulsory. However, compulsory fields risk inaccurate information being recorded. On 29/05/2023 our local health region made the indication field in prescriptions compulsory in the hospital prescribing system. This provided an opportunity to evaluate the effect of a compulsory indication field on prescriber behaviour. The text 'to be determined' was introduced in a drop-down selection box for use when the indication is unknown.
Aims. To evaluate making the indication field compulsory for all medicines in the hospital prescribing system.
Methods. The change in the proportion of prescriptions with an indication was compared for eight weeks after introduction of a compulsory indication field on 29/05/2023 to an equivalent eight-week period in 2022. Text in the indication field was manually classified as an indication, 'other text', 'rubbish text', 'to be determined', and blank. For prescriptions with 'to be determined' in the indication field, the proportion with an indication added, the dose changed, or the prescription ceased prior to discharge was measured.
Results. We analysed 81,646 prescriptions before and 83,427 after indications were made compulsory. The proportion of prescriptions with an indication increased from 29.2% to 78.1% ($p<0.01$). 'Other text' increased from 2.5% to 11.9% ($p<0.01$), 'rubbish text' from 0.0% to 2.7% ($p<0.01$) and 'to be determined' from 0.0% to 6.6% ($p<0.01$). Of 6,343 prescriptions with the indication 'to be determined' in the initial prescription, 5.6% were assigned an indication, 5.2% had the dose changed, and 20.8% were ceased, all prior to discharge.
Discussion. Introduction of compulsory indications for medicines increased recording indications in prescriptions substantially, with small increases in other text and rubbish text. Use of the 'to be determined' drop-down selection box rarely led to recording the indication prior to discharge and there was negligible effect on deprescribing.

P517. Casein kinase 1 delta inhibitor PF670462 has anti-tumorigenic activity in the triple negative breast cancer cell line, MDA-MB-231

Jana L Zielinski[1,2], Cameron J Johnstone[3], Alastair G Stewart[1,2]. Dept of Biochemistry & Pharmacology, Univ of Melbourne[1], ARC Centre for Personalised Therapeutics Technologies[2], Olivia Newton-John Cancer Research Institute[3], VIC, Australia.

Introduction. Casein Kinase 1 Delta (CK1δ) is a conserved serine/threonine protein kinase that is highly expressed in metastatic and primary breast tumour cells and involved in cellular processes, such as circadian rhythm, fibrogenesis and inflammation. Furthermore, there is emerging evidence that pro-inflammatory cytokines aid in metastasis. When CK1δ is knocked down or pharmacologically inhibited, breast tumour cell invasiveness and tumorigenicity is suppressed in cell lines and in orthotopic xenograft models. The dual CK1δ/ε inhibitor PF670462, which has off-target actions on JNK and $p38^{MAPK}$ is therefore a potential anti-cancer therapeutic agent.
Aims. To explore the anti-tumorigenic activity of PF670462 in a human breast tumour cell line.
Methods. MDA-MB-231 and CSNK1D shRNA knockdown cells were incubated with 3 μM of PF670462, the JNK inhibitor CC90001 or the $p38^{MAPK}$ inhibitor SB203580 for 48h. IL-1α (30 pM) or TGF-β (100 pM) induced IL-6, IL-8 and IL-11 cytokine levels were measured by immunoassay. Cell proliferation, viability and migration were measured with live cell microscopy in the presence and absence of PF670462 (1-10 μM). Global and phospho-proteomes were measured with LC-MS/MS Orbitrap Eclipse mass spectrometer.
Results. PF670462 and SB203580 attenuated the levels of IL-1α stimulated IL-6, IL-11 and IL-8 ($n=3$; $P<0.05$). PF670462 attenuated TGF-β stimulated IL-11 ($n=3$; $P<0.01$). 10 μM PF670462 or CSNK1D knockdown slows cell proliferation and migration ($n=3$;$P<0.001$). LC-MS/MS identified 7225 proteins in which pathways involved in cell migration and proliferation showed lower expression in CSNK1D knockdown cells.
Discussion. Cytokine-induced fibrogens and inflammagens may amplify fibrosis in breast cancer. Tumour fibrosis is associated with increased metastasis. Cytokine attenuation by PF670462 indicates therapeutic potential for metastatic forms of breast cancer. Moreover, inhibiting CK1δ may slow tumour cell progression. Therefore, CK1δ may provide a useful addition to therapeutic approaches for metastatic breast cancer.

P518. Influence of tumour microenvironment on the activity of breast cancer therapeutic agents

Xiaodan Zhang[1,2], Tianhong Cheng[1,2], Ellie Cho[1,3], Kalyan Shobhana[1,3], Paul McMillan[1,3], Alastair Stewart[1,2]. Department of Biochemistry and Pharmacology, The University of Melbourne[1], VIC, Australia; ARC Centre for Personalised Therapeutics Technologies[2], Melbourne, VIC, Australia; The Biological Optical Microscopy Platform (BOMP), The University of Melbourne[3], VIC, Australia.

Introduction. Emerging evidence suggests that the nutrient availability in the tumour microenvironment, the dimensionality in which *in vitro* tumour models are established and drug exposure profiles have crucial impacts on cellular processes, cell proteome and anti-cancer drug responses. Melbourne medium (MM), a plasma-like physiological medium developed by our group, is contrasted with conventional hyper-nutritional cell culture medium (CM) such as DMEM, for influence on the activity of anti-tumour agents.
Aims. Establish protein expression profiles in 2D and 3D in *in vitro* breast cancer models using MCF-7 and MDA-MB-231 cells and compare effectiveness of selected breast cancer drugs in different conditions.
Methods. The protein expression profiles in MDA-MB-231 and MCF7 cells in 2D and 3D conditions (MM vs. CM) were established by global proteomics. Acridine orange and ethidium bromide (AO/EB) staining was used in combination with Operetta high content imaging of viable and non-viable cell numeration in 2D conditions. Propidium iodide and Hoechst 33342 staining was used in combination with Zeiss LSM 900 Airyscan2 Confocal live cell imaging system to image spheroids (3D) in 384-well plates.
Results. In MDA-MB-231 cells, 284 and 71 proteins were found to be upregulated in CM compared to MM in 2D and 3D, respectively; 199 and 198 proteins were downregulated in CM compared to MM in 2D and 3D, respectively. The results for MCF-7 cells showed similar trends. In MCF-7 spheroids, paclitaxel and exatecan mesylate exhibit significantly different effectiveness profiles than in 2D.
Discussion. Protein expression is affected more by stiffness and/or dimensionality of culture than by medium composition. The effectiveness of paclitaxel and exatecan mesylate was greatly influenced by stiffness and/or dimensionality of the culture setting. Conventional indirect metabolic assessment for viability such as CellTiter-Glo (CTG) is subject to metabolic artefacts and was therefore replaced by direct enumeration of viable cells using live cell imaging confocal microscopy.

P519. Modulation of T- type Calcium Channel 3.1 by Cannabidiol (CBD) and its enantiomer +CBD.

Chris Bladen[1], Marina Santiago[1], Mark Connor[1].
Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, NSW[1]

Introduction: T-type calcium channels play critical roles in brain function and altered behavior due to injury or genetic mutations, can cause many diseases including pain and epilepsy[1,2]. Cannabidiol (CBD) is now approved in Australia for treatment of pain and epilepsy, but large knowledge gaps remain surrounding the exact mechanisms by which it exerts its therapeutic effect[3]. CBD inhibits T-type calcium channels, however, CBD is a chiral compound and its enanatiomers have been reported to have different effects at targets including CB1. It is not known whether the enantiomers of CBD modulate T-type channels similarly or otherwise.
Aims: To compare how (-) CBD, the naturally occurring compound and its enantiomer (+) CBD, regulate the T-type ion channel Cav3.1 that has been associated with epilepsy.
Methods: Patch clamp electrophysiology was used to compare the characterisitics of (-) CBD and (+) CBD modulation of Cav3.1. Experiments used HEK293 Flp-In T-REx cells stably expressing Cav3.1.
Results: (+) CBD blocked Cav3.1 more potently than (-) CBD, with an IC_{50} of 1.5 µM ± 0.05 µM, versus 3.4 µM ± 0.03 µM for (-) CBD. (-) CBD and (+) CBD produced similar negative shifts in activation (-5mV ± 0.7µM and -7mV± 0.8µM respectively) and both exhibited significant negative shifts in inactivation (-10 mV ± 0.2µM and -11 mV ± 0.3µM respectively) compared to vehicle control.
Discussion: Some phytocannabinoids, particularly cannabidiol, have shown to be efficacious in treating some severe forms of epilepsy such as Dravet syndrome, but the mechanism of action remains to be elucidated. Here we show that the unnatural/synthetic (+) enantiomer of CBD is more potent at modulating Cav3.1, a potentially important target for anti-epileptic drugs. This data is the first to show the inhibitory and kinetic effects of the (+) enantiomer of CBD on T-type channels, but whether the apparently increased modulatory effect of (+) CBD on Cav3.1 observed has any relevant pharmacological effect is yet to be determined.
1. Catterall WA et al (2005). *Pharmacol Rev* 57: 411-425. 2. Bladen, C et al., (2014). *ACS Chemical Neuroscience* 6: 277-287. 3. Devinsky O et al., (2014). *Epilepsia* 55: 791-802. 4. Mirlohi, S et al., (2022). *Br. J. Pharmacol.* 179: 4031–4043

P520. Calcium signaling and the breast cancer microenvironment

Farzaneh Forouz[1], Alice H. L. Bong[1], Krystyna A. Gieniec[2], Francisco Sadras[1], Mélanie Robitaille[1], Sarah J. Roberts-Thomson[1], Felicity M. Davis[1,2,3], Gregory R. Monteith[1]. School of Pharmacy, University of Queensland[1], Brisbane, QLD, Australia; School of Medical Sciences, The University of New South Wales[2], Sydney, Australia; Department of Biomedicine, Aarhus University[3], Aarhus, Denmark.

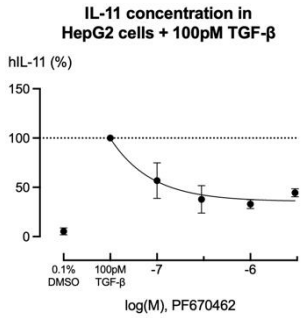
Introduction. Breast cancer is the second-leading cause of cancer-related fatalities among women in Australia. Calcium signaling is involved in various processes essential for tumor progression such as contributing to cell proliferation and invasion (Monteith et al, 2017). Signaling interactions occurring between cancer cells and fibroblasts within the tumor microenvironment play a pivotal role in activating cancer-associated fibroblasts (CAFs). CAFs contribute to critical processes such as metastasis and the development of therapeutic resistance. Recently, alterations in calcium influx in CAFs has been reported to be one of the features of breast cancer (Sadras et al, 2021).
Aims. To evaluate potential Ca^{2+} signalling cross-talk between cancer cells and CAFs in models of the breast cancer microenvironment.
Methods. In these studies, genetically encoded calcium sensors with distinct fluorescence spectral properties were employed to simultaneously assess cytosolic free Ca^{2+} levels in breast cancer cells and fibroblasts. Experiments were conducted using HMF3S human fibroblasts and breast cancer cells expressing the Ca^{2+} sensors GCaMP6m or JRCaMP1b. These cells were co-cultured in either two-dimensional (2D) or three-dimensional (3D) environments and assessed using automated epi-fluorescence imaging or confocal microscopy.
Results. Distinct spatial and temporal differences in cytosolic free Ca^{2+} changes were observed between fibroblasts and breast cancer cells after their activation in co-culture.
Discussion. These methodological advances offer new opportunities to gain deeper insights into calcium signaling in cancer-associated fibroblasts. The techniques developed could also help pinpoint specific calcium influx pathways to therapeutically target to inhibiting pathways important in breast cancer progression.

Monteith, G R et al (2017) Nature Reviews Cancer 17(6): 373-380
Sadras, F et al (2021) Biomedicines 9(6)

P521. Targeting casein kinase 1 delta/epsilon δin hepatocellular carcinoma

Gretel Happell[1], Daniel Tan[1,2], Alastair Stewart[1,2]. Department of Biochemistry and Pharmacology, The University of Melbourne[1], Melbourne, VIC, Australia; ARC Centre for Personalised Therapeutics Technologies[2], Melbourne, VIC, Australia.

Introduction. Inflammation and fibrosis are key precursors in the development of hepatocellular carcinoma. Inhibition of casein kinase 1 delta/epsilon (CK1δ/ε) with the dual inhibitor PF670462 has been shown to attenuate fibrosis *in vitro* and has anti-tumorigenic properties in a variety of tumours. The genes encoding for CK1δ/ε are upregulated in HCC and their expression are linked to a more aggressive tumour phenotype. Thus, CK1δ/ε represents a relevant target for treatment, as current treatments lack efficacy, resulting in high mortality from HCC globally (Zhu et al, 2022).
Aims. To elucidate the contribution of CK1δ/ε to inflammatory cytokines, fibrogenesis, and cell survival in an HCC model cell line, HepG2, to further assess its suitability as a target for treatment.
Methods. HepG2 cells were treated with PF670462 then stimulated with either TGF-β or IL-1α to induce inflammatory and fibrogenic cytokines. Cytokine levels were measured using ELISA, targeting interleukins IL-11, IL-6, and IL-8, and plasminogen activator inhibitor-1. Cell viability was quantified using trypan blue staining, and acridine orange/ethidium bromide fluorescent staining.



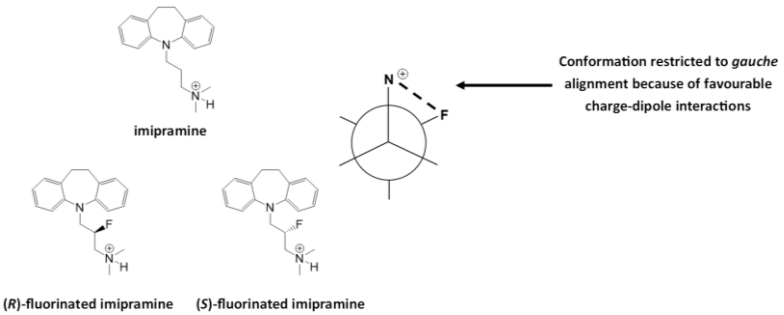
Results. PF670462 attenuated levels of fibrotic cytokines IL-11 and PAI-1 in HepG2 cells stimulated with TGF-β. IL-1α induced IL-8 levels were reduced by PF670462 pretreatment. PF670462 did not affect cell viability.
Discussion. These observations suggest that CK1δ/ε plays a role in inflammatory and fibrogenic cytokine production of HepG2 cells. CK1δ/ε may be a beneficial pharmacological target to treat the inflammation/fibrosis-driven HCC.

[1]Zhu M et al. (2022) Biol Proced Online. 24:21.

P522. Inducing conformational bias via fluorination to enhance selectivity and potency of imipramine.

Ishika Jaitly[1,2], Kinjal Patel[1], Luke Hunter[2], Nicola J. Smith[1]. School of Biomedical Sciences, Faculty of Medicine & Health, UNSW Sydney[1], Sydney, NSW, Australia. School of Chemistry, Faculty of Science, UNSW Sydney[2], Sydney, NSW, Australia.

Introduction. Tricyclic anti-depressants (TCAs) such as imipramine have commendable efficacy at the serotonin transporter (SERT), but their use is limited by off-target effects at muscarinic, histamine and adrenergic receptors (Feighner, 1999). The structural flexibility of the imipramine side chain could underlie its poor selectivity. We hypothesise that conformational preorganisation of this side chain via stereoselective fluorination, could have a two-fold benefit of (1) reducing off-target effects and (2) enhancing potency at SERT.



Aims. To test the effect of conformational restriction, mediated by stereoselective fluorination, on the selectivity and potency of TCAs, using imipramine as a model.

Methods. (*R*)- and (*S*)-fluorinated imipramine analogues were synthesised and confirmed to be enantiopure. An uptake and inhibition assay at SERT that makes use of the fluorescent dye, ASP+, was optimised and used to test the imipramine analogues. Time permitting, off-target effects will be tested using a NanoBiT G protein dissociation assay.

Results. ASP+ was demonstrated to be a substrate of SERT with a K_m of 13.34 µM (n = 3). Inhibition assays were then performed using this experimentally-derived K_m as a guide.

Discussion. It is expected that this study will determine whether conformational restriction of the imipramine side chain impacts the potency and selectivity of imipramine for SERT.

Feighner JP (1999) J Clin Psychiatry 60:4-11

P523. Developing nanobodies as subtype-selective α_{1A} adrenergic receptor tools

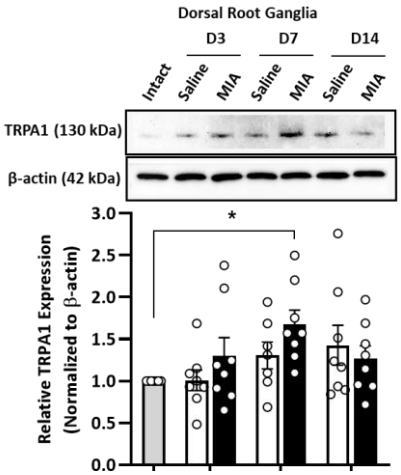
Saund Yadanar[1], Riley Cridge[1,3], Arisbel Batista[2], Nicholas Veldhuis[2], Christopher Draper-Joyce[1,3], Daniel J. Scott[1,3]. Drug Discovery Innovation, The Florey Institute[1], Melbourne, VIC, Australia; Integrated Neurogenic Mechanisms, Monash Institute of Pharmaceutical Science[2], Melbourne, VIC, Australia. LASEREDD Therapeutics, Melbourne, VIC, Australia

α_1-adrenergic receptors (α_1-AR) belong to class A G protein-coupled receptor (GPCR) and play an essential role in mediating the physiological function of noradrenaline through the sympathetic nervous system. The lack of subtype-specific tools for the three subtypes of the α_1-AR (α_{1A}, α_{1B} and α_{1D}) has long been a bottleneck to studying the roles and distribution of the specific subtypes and has hindered target validation of their therapeutic potential. Nanobodies or single-domain antibody fragments (sdAbs) possess the specificity of monoclonal antibodies and elongated CDR3s that enable access into cryptic drug pockets of GPCRs. Herein, a panel of α_{1A}-AR specific nanobodies were identified from an immune repertoire of a hyper-immunised alpaca through mammalian cell display library enrichment and high-throughput screening of single clones. Subsequently, nanobodies with subtype selectivity (α_{1A}-AR vs α_{1B}-AR) and species cross-reactivity (human & rat) have been identified and characterised. These nanobodies represent the first tools to validate the distribution and roles of this target receptor in pathophysiology. Similar workflows can now be employed to identify nanobodies for other class A GPCRs and membrane proteins for broad research purposes, including structure biology, imaging, and diagnostics.

P524. TRPA1 expression in joints and DRGs of monoiodoacetate-induced rat knee OA pain

Scarlett Desclaux[1], Orada Sriwatananukulkit[1], Pongsatorn Meesawatsom[2], Ruedee Hemstapat[1]. Dept of Pharmacol, FCs of Sci, Mahidol Univ[1], BKK, Thailand; Dept of Pharmacol, FCs of Pharmacy, Mahidol Univ[2], BKK, Thailand.

Introduction. Transient receptor potential ankyrin 1 (TRPA1) has been implicated as a novel target of pain (Koivisto et al, 2014). However, a relationship of time course changes in TRPA1 expression with pain behaviour and structural changes of joint tissues after injection of monoiodoacetate (MIA) has not been characterized.
Aim. To investigate the time course changes in the expression of TRPA1 during early and chronic phase of MIA-induced model of osteoarthritis (OA) pain.
Methods. General anaesthesia was administered by inhalation of 3-5% isoflurane. OA was induced by intraarticular injection of 2 mg MIA. Pain-like behaviour was assessed by mean of static weight bearing for 2 weeks post-OA induction. Knee joint tissues and L3-L4 dorsal root ganglia (DRGs) were collected on day 3, 7 and 14 for TRPA1 expression analysis using both western blot and immunohistochemistry. Knee joint histopathology was also scored.
Results. The development of MIA-induced pain-like behavior was confirmed in this model, in which the most painful day was observed at day 3 and persisted to at least 14 days post-induction. A significant increase TRPA1 expression in DRGs was observed on day 7 by 1.7 ± 0.16 (n = 8, P<0.03) when compare to the intact animals and declined on day 14. This study also revealed that TRPA1 expression in the joint tissues increased in synchrony with histological severity.



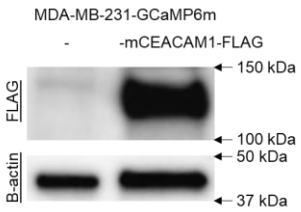
Discussion. The present finding suggested that TRPA1 may contribute in part to pain-like behaviour observed in the early phase of MIA-induced knee OA pain. However, further study is required to investigate whether TRPA1-specific antagonist could be effective in alleviating OA knee pain.

Koivisto A et al (2014) Basic Clin Pharmacol Toxicol. 114:50-55

P525. Developing a human cell model to study calcium signalling during coronavirus infection.

Shao Ming Chan[1], Gregory Monteith[1], Sarah Roberts-Thomson[1], Larisa Labzin[2], Mélanie Robitaille[1]. School of Pharmacy, The University of Queensland[1], St Lucia, QLD, Australia; Institute for Molecular Bioscience, The University of Queensland[2], St Lucia, QLD, Australia.

Introduction. Mouse hepatitis virus (MHV) is a rodent coronavirus of the *Betacoronavirus* genus. MHV-1 serves as a surrogate model for human coronaviruses related to severe acute respiratory syndrome (SARS) (Körner R et al 2020). MHV infection requires binding of the spike protein to the MHV entry receptor mCEACAM1, a protein that humans do not express. Understanding calcium signaling during MHV-1 infection in host cells can provide insights into new therapeutic targets.
Aims. To generate a human cell line expressing the genetically encoded calcium indicator GCaMP6m and mCEACAM1 protein and assess its susceptibility to MHV-1 infection.



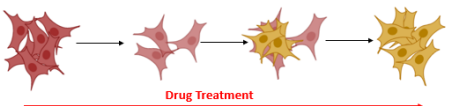
Methods. mCEACAM1 cDNA with a FLAG tag was cloned into a lentiviral compatible vector. Using a second-generation lentiviral system, mCEACAM1-FLAG was transduced in MDA-MB-231 cells stably expressing GCaMP6m, previously produced by Bassett JJ et al (2018). Protein expression of mCEACAM1-FLAG was validated by immunoblotting and immunofluorescence. MHV-1 (ATCC VR-261) was propagated in NCTC clone 1469 cells (ATCC CCL-9.1) and harvested as a crude supernatant. MDA-MB-231-GCaMP6m-mCEACAM1-FLAG cells were mock- or MHV-infected.
Results. The generation of a stable MDA-MB-231-GCaMP6m cell line expressing mCEACAM1 protein with evidence of the expected plasma membrane localisation was successful. This cell line was susceptible to MHV-1 infection and exhibited distinct cytopathic effects including cell death and formation of syncytia, defined as multinucleated cells due to cell-cell fusions at 10 h post infection.
Discussion. The developed coronavirus infectable model provides opportunities to assess cell signalling during viral-host interaction.

Bassett JJ et al (2018) Cell Calcium 72:39-50.
Körner R et al (2020) Viruses 12;12(8):880.

P526. Exploring calcium signalling remodelling during adaptive cancer drug tolerance

Mélanie Robitaille[1], Helmut Schaider[2], Sarah Roberts-Thomson[1], Gregory Monteith[1]
School of Pharmacy[1], The University of Queensland, Woolloongabba, QLD, Australia. Frazer Institute[2], The University of Queensland, Woollongabba, QLD, Australia.

Introduction. Adaptive tolerance to molecularly targeted therapies is a major limitation to the long-term success of the treatment with many cancer patiens replasing after an initial response. Remodelling of various cellular signalling pathways may be associated with the development of various forms of resistance to cancer therapies (Shaffer et al.).



	Parental	Early tolerance	Late tolerance	Resistance
Drug Sensitivity	Sensitive	Tolerant	Tolerant	Resistant
Reversibility state	N/A	Reversible	Reversible	Irreversible
Proliferation	Proliferative	Slow cycling	Mix	Proliferative
Stemness	Low	Very High	High	Low
Calcium signalling remodelling	Normal	↑	↑↑	↑

Aims. Our study aimed to assess calcium signalling remodelling in adaptive drug tolerance models.
Methods. Melanoma and lung cancer cells were treated with a sublethal dose of molecularly targeted therapy drugs for several days/weeks to initiate adaptive drug tolerance. Remodelling of calcium signalling components was assessed by RT-qPCR.
Results. Our results indicate that alterations in specific members of the calcium signalling toolkit are a feature of tolerance to some molecularly targeted cancer therapies. Current studies are now defining the role of these calcium signaling components in the establishment and/or maintenance of tolerance to molecularly targeted cancer therapies.
Discussion. Despite the superior efficacy and reduced toxicity of molecularly targeted therapies, their effectiveness is limited by adaptive drug tolerance. Overcoming cancer drug tolerance through targeting specific aspects of calcium signaling is a promising area warranting further investigation.

This project is funded by Tour de Cure, Pioneer grant.

Shaffer SM et al. (2017) Nature 546(7658):431

P527. Assessing gene expression of pannexin isoforms in breast cancer cell lines

Monami Ghosal[1], Alice HL Bong[1], Sarah J Roberts-Thomson[1], Gregory R Monteith[1]. School of Pharmacy, University of Queensland[1], Woolloongabba, QLD, Australia

Introduction: Triple negative breast cancer (TNBC) has the poorest prognosis of the breast cancer subtypes, and lack specific therapeutic targets. Proteins regulating calcium signalling are potential cancer therapeutic targets. Pannexins are ATP-permeable channels widely expressed throughout the body. Recently, pannexin-1 was shown to be a novel regulator of calcium entry in neurons (Patil *et al.,* 2022), suggesting that pannexin-1 and/or other isoforms (pannexin-2 and -3) may also regulate calcium signalling in other cells. However, there are few studies on the expression levels of pannexin isoforms in breast cancer cells, and whether there is any correlation with breast cancer subtypes.
Aims: To assess the gene expression of pannexin isoforms (pannexin-1, pannexin-2 and pannexin-3) in breast cancer cell lines and explore any association between pannexin isoform expression and breast cancer subtypes.
Method: To determine a suitable housekeeping gene, the Ct values of four commonly used genes (18s, GAPDH, ACTB and PGK1) were determined in seven breast cancer cell lines - representing luminal, HER2 positive, and TNBC subtypes - using quantitative real-time PCR. Expression levels of pannexin 1, 2 and 3 isoforms in these cell lines were then assessed using quantitative real-time PCR using the selected housekeeping gene to determine delta Ct (dCt) values.
Results: GAPDH showed the lowest variability in its Ct value (SD = 0.932) and was chosen as the housekeeping gene for quantitative real-time PCR studies. All pannexin isoforms were expressed in all studied breast cancer cell lines. Pannexin-1 showed the most variation in expression between the different cell lines. However, no significant association was found between the expression levels of any of the pannexin isoforms and breast cancer subtype.
Discussion: This study confirmed that pannexin 1, 2 and 3 channels are expressed in widely used breast cancer cell lines. Given recent findings showing a role for some pannexins in cancer (Jalaledine *et. al.* (2019)), more studies are required to further delineate the association between pannexin isoforms, calcium regulation and specific cancer hallmarks in breast cancer.

Jalaledine N. *et. al.* (2019) Cancers, 11, 1967
Patil C. S. *et. al.* (2022) PNAS, 119

P528. 8-Shogaol suppresses rheumatoid arthritis by direct inhibition of TAK1

Yong Joon Lee[1], Seongin Jo[1], Snigdha Samarpita[2], Ji Su Lee[1], Joe Eun Son[3], Minju Jeong[4], Jae Hwan Kim[1], Seungpyo Hong[5], Seung-Ah Yoo[6], Wan-Uk Kim[7], Seungki Lee[8], Youn Kyoung Son[8], Byoung-Hee Lee[8], Mahaboobkhan Rasool[2], Sanguine Byun[1]. Department of Biotechnology, Yonsei University[1], Seoul, Republic of Korea; School of Bio Sciences and Technology, Vellore Institute of Technology (VIT)[2], Vellore, Tamil Nadu, India; Program in Developmental & Stem Cell Biology, The Hospital for Sick Children[3], Toronto, Canada; Department of Agricultural Biotechnology, Seoul National University[4], Seoul, Republic of Korea; Food Functionality Research Division, Korea Food Research Institute[5], Wanju-gun, Jeollabuk-do, Republic of Korea; Department of biomedicine and health sciences, The Catholic University of Korea[6], Seoul, Republic of Korea; Division of Rheumatology, Department of Internal Medicine, Catholic University of Korea[7], Seoul, Republic of Korea; National Institute of Biological Resources[8], Seo-gu, Incheon, Republic of Korea

Introduction. Rheumatoid arthritis involves chronic immune-mediated joint inflammation and damage. Targeting fibroblast-like synoviocytes (FLS), central to the disease's development, offers a promising therapeutic avenue for addressing challenges in achieving remission and preventing disability.
Aims. The objective of this study was to investigate the potential of 8-Shogaol as an inhibitor of rheumatoid arthritis (RA) and elucidate its mechanism of action.
Methods. From screening 315 natural extracts for potent inhibition of IL-17-mediated IL-6 production. *Zingiber officinale* extract emerged as the top hit, with 8-shogaol identified as the active compound. In vitro, 8-shogaol was tested in RA patient-derived FLS for its anti-inflammatory effects, elucidating its impact on key RA signaling pathways. In vivo, an RA animal model was utilized to assess the therapeutic efficacy of 8-Shogaol.
Results. 8-Shogaol demonstrated significant inhibition against TNF-α-, IL-1β-, and IL-17-mediated inflammation and migration in RA-FLS and the 3D synovial culture system. 8-shogaol directly targeted and selectively inhibited the activity of TAK1, leading to suppression of downstream signaling pathways including IKK, Akt, and MAPK. In the AIA rat model, treatment with 8-shogaol reduced paw thickness and improved walking performance. Furthermore, 8-shogaol reversed joint structural pathologies in AIA rats and decreased inflammatory biomarkers in the joints.
Discussion. These findings suggest the potential of 8-Shogaol as a promising candidate for the RA therapeutics, offering new insights for RA treatment by reversing pathologies of the inflamed synovium through TAK1 inhibition.

P529. Gene expression of calcium signalling modulators in SARS-CoV-2 infection

Mia C Akison[1], Kathrin Eschke[2], Gregory Monteith[1], Sarah Roberts-Thomson[1], Larisa Labzin[2], Mélanie Robitaille[1]. School of Pharmacy, The University of Queensland[1], Brisbane, QLD, Australia; Institute for Molecular Biosciences, The University of Queensland[2], Brisbane, QLD, Australia.

Introduction. Calcium is an essential second messenger involved in a plethora of physiological processes from maintaining cell growth and proliferation to energy metabolism, making the homeostasis of intracellular calcium vital for cell survival. Viruses hijack the host cell's calcium modulators to regulate viral entry, gene replication, maturation, and release, therefore causing calcium signalling to be a commonly dysregulated pathway in viral infections (Saurav et al, 2021). Following the recent COVID19 pandemic, the role of calcium in SARS-CoV-2 infection is being increasingly studied. However, how SARS-CoV-2 dysregulates calcium signalling during infection is not fully understood.
Aims. The primary aim of this study was to identify calcium-related genes modified by SARS-CoV-2 infection.
Methods. A549 human lung epithelial cells transduced with ACE-2 receptors were either left uninfected as a control or were infected with SARS-CoV-2 QLD02 virus and collected at time points 1-, 24-, 48- and 72-h post infection. cDNA from each sample was reverse transcribed and a screen of 42 calcium-related genes was conducted using RT-qPCR. The relative gene expression was determined for each gene using the comparative C_T method.
Results. Changes in calcium signalling genes were observed.
Discussion. By completing a screen of genes involved in calcium signalling, this research will lay the groundwork for identifying calcium-related genes modified by SARS-CoV-2 infection, serving as a steppingstone for future research into mechanisms surrounding SARS-CoV-2 infection pathways.

Saurav S et al (2021) Mol Aspects Med 81:101004

P530. Combining LC-MS/MS with bioinformatic approaches for understanding bioactivities of propolis

Eun Heo[1], Ye-Ryeong Cho[1], Kyeong Ah Jo[2], Soo-yeon Park[2], Jae-won Choi[3], Gwangmin Kim[4], Tae Yeon Kim[4], Soohwan Lee[5], Sung-kuk Kim[6], Doheon Lee[4], Seokwon Lim[5], Soon Ok Woo[6], Seungki Lee[7], Youn Kyoung Son[7], Byoung-Hee Lee[7], Sanguine Byun[1,*], and Ji Yeon Kim[2,*]. Department of Biotechnology, Yonsei University[1], Seoul, Republic of Korea; Department of Food Science and Technology, Seoul National University of Science and Technology[2], Seoul, Republic of Korea; Department of Physical Education, Yonsei University[3], Seoul, Republic of Korea; Department of Korea Advanced Institute of Science and Technology[4], Daejeon, Republic of Korea; Department of Food Science and Biotechnology, Gachon University[5], Seongnam-si, Gyeonggi, Republic of Korea; Department of Agrobiology, Division of Apiculture, National Institute of Agricultural Sciences[6], Wanju-gun, Jeollabuk-do, Republic of Korea; National Institute of Biological Resources[7], Seo-gu, Incheon, Republic of Korea

Introduction. We employed network biology and cheminformatic approaches to predict new target diseases, active components of propolis. Applying LC-MS/MS analysis results of propolis to Context-Oriented Directed Associations (CODA) and Combination-Oriented Natural Product Database with Unified Terminology (COCONUT) systems indicated atopic dermatitis as a novel target disease.
Aims. The objective of this study was to predict propolis-associated immune diseases by combining LC-MS/MS and CODA/COCONUT and investigate therapeutic effects of propolis against atopic dermatitis (AD).
Methods. Propolis was collected in Korea and extracted in 80% ethanol solution. The phenolic compounds present in propolis were analyzed using LC-MS/MS. Potential target disease of propolis was analyzed using CODA and COCONUT system. AD related gene expression and cytokine secretion were measured in vitro.
Results. 17 compounds have been identified in propolis by LC-MS/MS. The CODA/COCONUT analysis showed that 12 out of the 17 compounds of propolis are potential active compound against AD. Propolis treatment significantly decreased AD related inflammatory cytokines and chemokines secretion in AD-like model. Through CODA/COCONUT analysis, 10 genes were selected was expected to affect AD by being regulated by many propolis components. Propolis treatment decreased most of the selected gene expression.
Discussion. Theses finding suggest the Korean propolis is a potential therapeutic natural agent for the treatment of AD and the CODA/COCONUT system is worthy of further application to predict the bioactivity of other foods.

P531. Selective killing of mTORC1-hyperactive cancer cells through targeting the RUVBL1/2-TTT pathway

Ji Su Lee[1 *], Seung Ho Shin[2*], Jia-Min Zhang[3 *], Zarko V. Boskovic[4], Ran Zhao[5], Mengqiu Song[5], Rui Wang[5], Jie Tian[5], Mee-Hyun Lee[5], Jae Hwan Kim[1], Seungki Lee[6], Youn Kyoung Son[6], Byoung-Hee Lee[6], Lee Zou[3,7], Sanguine Byun[1,8†]. Department of Biotechnology, Yonsei University[1], Seoul, Korea; School of Bio Sciences and Technology, The Hormel Institute, University of Minnesota[2], Austin, USA; Massachusetts General Hospital Cancer Center[3], Charlestown, USA; Broad Institute of MIT and Harvard[4], Cambridge, USA; School of Basic Medical Sciences, Zhengzhou University[5], Zhengzhou, Henan, China; Seoul, Korea; National Institute of Biological Resources[6], Incheon, Korea; Department of Pathology, Harvard Medical School[7], Boston, USA; Cutaneous Biology Research Center, Massachusetts General Hospital and Harvard Medical School[8], Charlestown, USA;

Introduction. The mTOR inhibitor, rapamycin and its analogs have demonstrated anticancer efficacy in preclinical models. Despite the initial positive response in some tumors that have high mTOR activity, the tumors re-grow after discontinuation of therapy. An unmet need therefore exists for the development of combination therapies or new approaches that can elicit cytotoxic responses in mTOR-hyperactive cancers.
Aims. we screened for small molecules targeting cells with a hyperactive mTORC1 phenotype.
Methods. We designed screening compounds displaying selective cytotoxicity in high-mTORC1 cells by measuring the capability to induce cell death rather than to reduce cell proliferation.
Results. The sensitivity of PL(piperlongumine) highly depends on mTORC1 activity. The addition of PL to cells followed by immunoprecipitation with Flag-Tel2 demonstrated a decreased interaction of Tel2 with TTI1 and RUVBL1, the components of the RUVBL1/2-TTT complex, indicating that PL caused disassembly of the RUVBL1/2-TTT complex. We found that PL causes increase of γ H2AX in the cells. After DNA damage, the initial phosphorylation of γ H2AX could be delayed due to PL-induced decrease of ATM and ATR. We also found that increasing mTORC1 activity resulted in upregulation of c-Myc. our results show that high mTORC1 activity have upregulated levels of c-Myc and DNA damage stress, generating overreliance on RUVBL1/2 to maintain cellular integrity.
Discussion. Our findings highlight the potential advantage of targeting RUVBL1/2 for selective killing of cancer cells addicted to the mTOR pathway and suggest a therapeutic strategy for a biomarker-based personalized treatment.

P532. Hydroxychavicol inhibits the expression and activity of MMP-9 induced by RANKL in osteoclasts.

Wisutthaporn Inthanop[1], Sirada Srihirun[2], Chareerut Phruksaniyom[2], Pornpun Vivithanaporn[3].
Faculty of Medicine Ramathibodi Hospital, Mahidol University[1], Bangkok, Thailand.
Department of Pharmacology, Faculty of Dentistry, Mahidol University[2], Bangkok, Thailand.
Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University[3], Samut Prakarn, Thailand.

Introduction. Matrix metalloproteinase-9 (MMP-9), a gelatinase/type IV collagenase, plays a key role in osteoclasts' bone resorption activity by enhancing the degradation of bone matrix. The inhibition of MMP-9 by specific MMP-9 inhibitors or gene knockout attenuated osteoclast formation and activity. Our preliminary study showed that 10 μM hydroxychavicol, a non-toxic concentration, inhibited RANKL-induced osteoclatogenesis in RAW264.7 cells.
Aims. This study aimed to investigate the effect of hydroxychavicol, an active phenol compound in betel leaves (Piper betle), on RANKL-induced MMP-9 expression and activity in RAW264.7.
Methods. RAW264.7 cells were treated with 10 μM of hydroxychavicol and 50 ng/mL RANKL for 4 days. MMP-9 mRNA expression and enzymatic activity were determined by real-time polymerase chain reaction and gelatin zymography.
Results. Hydroxychavicol (10 μM) caused 68 and 36% inhibition of RANKL-induced MMP-9 mRNA expression and activity, respectively.
Discussion. Hydroxychavicol inhibits osteoclastogenesis by suppressing the expression and activity of MMP-9. However, hydroxychavicol's mechanism of action on osteoclastogenesis needs further investigation.

P533. Hydroxychavicol inhibits RANKL-induced expression of osteoclast-specific genes.

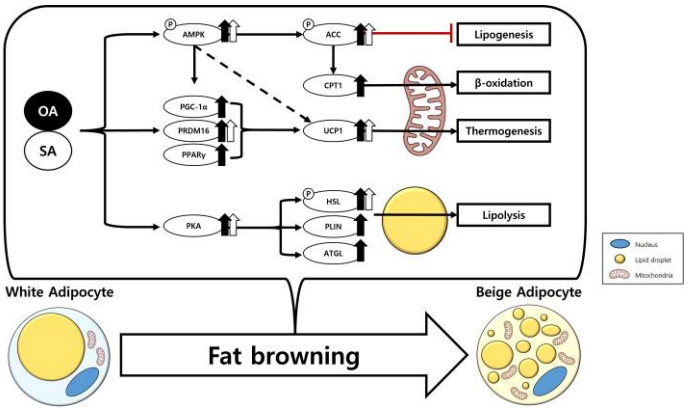
Pitchanun Kongphanich[1], Sirada Srihirun[2], Chareerut Phruksaniyom[2], Pornpun Vivithanaporn[3]
[1]Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.
[2]Department of Pharmacology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand.
[3]Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakarn, Thailand.

Introduction. Hydroxychavicol, a major active phenolic compound from the leaves of *Piper betle*, previously inhibited bone loss by promoting bone formation in rats. Our preliminary study demonstrated that hydroxychavicol suppressed RANKL-induced TRAP-positive multinucleated cells and F-actin ring formation in RAW264.7 cells. The expression of osteoclast-specific genes, including cathepsin K, V-ATPase and dendritic cell-specific transmembrane protein (DC-STAMP) plays a key role for osteoclast maturation. Cathepsin K and V-ATPase are bone-resorptive enzymes, while DC-STAMP is a protein for the fusion of osteoclast precursor cells.
Aims. This study aimed to investigate the effect of hydroxychavicol on RANKL-induced expression of cathepsin K, V-ATPase and DC-STAMP by real-time polymerase chain reaction (PCR).
Methods. RAW264.7 cells were treated the non-toxic concentration of hydroxychavicol (1.25, 2.5, 5, and 10 μm) and 20 ng/mL for 4 days (The half maximum inhibitory concentration of hydroxychavicol on RAW234.7 cells was 37 μM).
Results. Hydroxychavicol caused concentration-dependent decreased expression of cathepsin K and DC-STAMP induced by RANKL. However, hydroxychavicol did not affect the expression of v-ATPase induced by RANKL.
Discussion. Hydroxychavicol inhibits osteoclastogenesis by suppressing RANKL-induced expression of osteoclast-specific genes.

ASCEPT Annual Scientific Meeting
Transformational Pharmacology & Toxicology
20–23 NOV 2023 International Convention Centre, Sydney
#ASCEPT2023
www.asceptasm.com

P534. Oleanolic acid and syringic acid induce fat browning through UCP1 activation

Ho Seon Lee, Sung Ho Lim, Seung Min Choi, Gayoung Choi, Chang-Ik Choi.
Integrated Research Institute for Drug Development, College of Pharmacy, Dongguk University-Seoul, Goyang 10326, Republic of Korea.



Introduction. In recent modern society, the number of obese patients is dramatically increasing around the world. The induction of white adipocytes to a brown adipocyte-like phenotype is called fat browning, a novel strategy to increase energy expenditure to ameliorate obesity. Moreover, natural bioactive products to treat obesity have attracted a lot of attention because of the various side effects of synthetic anti-obesity drugs. Oleanolic acid (OA) and syringic acid (SA), naturally-derived compounds used in our experiments are easily found in olives. Although both compounds have previously been reported to have anti-obesity effects, little is known about their mechanisms of fat browning.

Aims. We investigated the effects of OA and SA on fat browning and underlying mechanisms in 3T3-L1 adipocytes.

Methods. The MTT assay was used to determine cell viability, and Oil-Red-O staining was performed to measure changes in lipid accumulation in 3T3-L1 cells. The western blot assay was performed to confirm the protein expression, and quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine the mRNA expression. For immunofluorescent staining, uncoupling protein 1-fluorescein isothiocyanate (UCP1-FITC) antibody was applied and stained with MitoTracker Red and 4'-6-diamidino-2-phenylindole (DAPI). All the data are expressed as the mean ± SEM (n=3). Student's t-test was used to determine the difference between the means.

Results. Both compounds did not affect cell viability and reduced lipid accumulation within the tested concentrations (1-8 μg/mL). These compounds have been shown to increase the expression of the thermogenic marker UCP1 and to suppress lipogenesis by activating adenosine monophosphate-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC). The OA increased the expression of thermogenic markers proliferator-activated receptor gamma (PPARγ), PPARγ coactivator 1-alpha (PGC-1α) and PR domain-containing protein 16 (Prdm16). In addition, OA enhanced the expression of lipolysis markers hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), perilipin (PLIN), and protein kinase A (PKA), and SA increased HSL and PKA. The expression of all beige-specific gene was increased in OA, while partial activity was observed in SA. Interestingly, SA decreased both adipogenic markers PPARγ and CCAAT/enhancer-binding protein alpha (C/EBPα), but those were increased OA.

Discussion. These study results show the potential of SA to role as a potent anti-obesity agent, while OA enhanced thermogenesis pathway. Moreover, OA showed the possibility of regulating UCP1 expression through the AMPK pathway. We confirmed that both natural compounds (OA and SA) have fat browning effects as promising anti-obesity agents. The browning effect was found to be more potent with OA than with SA.

P535. The effect of *C. racemosa* extract on anti-wrinkle and anti-melanogenesis in skin cells

Sukprasert S[1], Janjadkarn A[1], Treveeravoot S[1] ,Sangpairoj K[2], Vivithanaporn P[3], Siangcham T[1] Faculty of Allied Health Sciences, Burapha University[1], Chon Buri, Thailand; Division of Anatomy, Faculty of Medicine, Thammasat University[2], Pathumthani, Thailand; Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University[3], Samut Prakan, Thailand.

Introduction. *Caulerpa racemosa* is macroalgae found mostly on the coast of Southeast Asia, especially in the Gulf of Thailand. Flavonoids are enriched in this algae that provide anti-melanogenesis and anti-aging properties, making them useful as cosmetic agents.
Aim. We test the cytotoxicity of the *C. racemosa* in ethanol extract and the underlying mechanism in anti-wrinkle and anti-melanogenesis in human keratinocyte and melanocyte cells.
Methods. The toxicity of the *C. racemosa* in ethanol extract on cell viability of HaCaT human keratinocyte cells and SK-MEL-5 human melanoma cells tested by MTT assays at 24h. The expression of mRNA and proteins related to wrinkle formation and melanogenesis were measured using real-time PCR and western blot analysis.
Results. CRET at 200 µM reduces cell viability of HaCaT cells by 77.88 ± 3.44 % and SK-MEL-5 cells by 68.06 ± 0.60% µM. For 24h The IC50 of CRET in HaCaT cells was 398.4 ± 0.80 µM and SK-MEL-5 was 233.7 ± 0.60 µM. CRET concentration 100 µM suppressed the expression of MMP-1 and MMP-2 and increased ERK expression in HaCaT cells. The MITF and p38 MAPK expression were decreased at final concentrations 100 µM CRET-treated SK-MEL-5 cells.
Discussion The present study showed that CRET not toxic to human keratinocyte and melanocytes in low concentrations. CRET downregulation of MMP-1 and MMP-2, which may related to anti-wrinkle effect. The decreased expression of marker of melanogenesis by CRET may result to anti-melanogenesis effect in human melanocyte

P536. Exploring molecular determinants underpinning high potency of MIPS3215 at adenosine A_{2B} receptor

Cam Sinh Lu[1], Ee Von Moo[1], Jon Kyle Awalt[2], Peter J Scammells[2], Lauren T May[1], Anh TN Nguyen[1]. Drug Discovery Biology[1] and Medicinal Chemistry[2], MIPS, Monash University, Parkville, VIC, Australia.

Introduction. The adenosine A_{2B} receptor ($A_{2B}R$) is a pivotal therapeutic target for a range of diseases, including ischemia, myocardial infarction, cancer, and fibrosis (Vecchio et al, 2019). As endogenous adenosine has low affinity for the $A_{2B}R$, the majority of $A_{2B}R$ effects occur during stress or injury, which is typically associated with elevated local adenosine levels. There is a paucity of potent, selective, and high-efficacy $A_{2B}R$ agonists. We recently identified a selective $A_{2B}R$ agonist with the highest known potency, MIPS3215 (Awalt et al, 2022). MIPS3215 can provide key insights into the molecular determinants that underpin $A_{2B}R$ efficacy and selectivity and as such facilitate drug discovery efforts at this potential therapeutic target.
Aim. To elucidate the molecular basis of high $A_{2B}R$ potency of MIPS3215 using a combination of computational and pharmacological approaches.
Methods. Computational docking of MIPS3215 was performed using an active $A_{2B}R$ structure (PDB ID: 7XY7). Key mutant $A_{2B}Rs$ containing single alanine substitutions were stably expressed in FlpINCHO cells. ELISA, cAMP accumulation and intracellular calcium mobilisation assays were performed to quantify the changes in receptor expression and functional potency and efficacy of NECA, a non-selective $A_{2B}R$ agonist, and MIPS3251.
Results. Among 24 mutants tested, only four significantly reduced receptor expression level. Alanine mutations of non-conserved residues located in the top of transmembrane 7 (N273A) and extracellular loop 3 (K265A and K267A) critically impacted MIPS3215 potency and selectivity at the $A_{2B}R$, leaving NECA unaffected. Docking simulation predicted that the linker and allosteric moiety of MIPS3251 interact with these residues. Notably, K265A significantly changed the bias profile of MIPS3251, suggesting the role of the extracellular loop 3 in biased agonism at the $A_{2B}R$.
Discussion. This study has identified critical amino acid residues for the high potency and selectivity of MIPS3215 at the $A_{2B}R$, contributing to a profound understanding of an efficient probe for the investigation of the $A_{2B}R$.

Awalt JK et al (2022) J Med Chem 65:9076-9095.
Vecchio EA et al (2019) Pharmacol Ther 198:20-33.

P537. Developing an optogenetic system to shine a light on intracellular GPCR signalling

Chantel Mastos[1], Alexandra-Madelaine Tichy[2], Christina G Gangemi[2], Andrew M Ellisdon[3], Harald Janovjak[4], Michelle L Halls[1]. Drug Disc Biol Theme, Monash Inst Pharm Sci[1], Monash Uni, Parkville, VIC, Australia; Aust Regen Med Inst[2] and Dept Biochem & Mol Biol, Biomed Discov Instit[3], Monash Uni, Clayton, VIC, Australia; Flinders Health and Medical Research Institute[4], College of Medicine and Public Health, Flinders Uni, SA, Australia.

Introduction. G protein-coupled receptors (GPCRs) mediate vastly diverse responses. Accumulating evidence reveals they can also signal from intracellular membranes. Differential localisation of GPCRs at the cell surface or intracellular membranes may result in location-specific outcomes. A fundamental understanding of localised GPCR signalling is required, however several pharmacological methods (e.g., endocytic inhibitors) can have confounding consequences. We therefore turned to optogenetic methods.
Aims. Establish a targeted optogenetic rhodopsin β2-adrenoceptor chimera (opto-β2AR) (Siuda et al., 2015) in human embryonic kidney (HEK) 293 & human highly metastatic (HM) triple negative breast cancer, MDA-MB-231 HM, cells. Quantify light-mediated cAMP and ERK phosphorylation (pERK).
Methods. Opto-β_2AR was targeted to intracellular membranes using literature-sourced location sequences. Targeting was confirmed with confocal microscopy, line scan analysis and a custom ImageJ script. Light-mediated receptor signalling was quantified using cAMP and pERK assays.
Results. Opto-β_2AR was successfully targeted to early endosomes, Golgi and nucleus. In both cell lines, opto-β_2AR activation modulated pERK levels in opposite directions (fig. 1, n=4, error bars mean±SEM), analogous to its wild-type counterpart. There were also light-dependent cAMP increases in both cell lines with differential effects based on location.
Discussion. Light-activated targeted opto-β_2ARs can be used to understand location-specific GPCR signalling. Future studies will use this system to investigate a mechanistic basis as disease-relevant GPCR signalling may also be location-dependent. A greater understanding of this is likely to encourage new strategies for GPCR-targeted drug discovery.

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

P538. Exploring A_{2B} receptor regulated human cardiac fibroblast phosphoproteome for fibrosis target discovery

Joel W Coffie[1], Bui San Thai[1], Paul J White[1], Simon R Foster[2], Lauren T May[1]. Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences[1], Monash University, VIC, Australia; Cardiac Bioengineering, QIMR Berghofer Medical Research Institute[2], QLD, Australia.

Introduction. There remains unmet therapeutic need to treat pathological cardiac fibrosis to reduce heart failure progression. The A_{2B} receptor, a G protein-coupled receptor, can modulate second messengers and decrease the expression of transforming growth factor beta 1 (TGF-β1), a pro-fibrotic mediator, in cardiac fibroblasts (Vecchio et al, 2016). Mapping the complex downstream phosphorylation events following A_{2B} receptor activation in human cardiac fibroblasts will refine our understanding of the potential of the A_{2B} receptor as a therapeutic target to treat fibrosis.
Aim. To delineate the global phosphorylation networks downstream of A_{2B} receptor activation in adult human ventricular cardiac fibroblasts (CFs) in the presence or absence of the pro-fibrotic mediator, TGF-β1.
Methods. Quantitative data-dependent acquisition mass spectrometry (DDA-MS) phosphoproteomics was employed to map the global protein phosphorylation events in CFs (Lonza, Switzerland) following agonist exposure, NECA (7 min; 0.28 μM) ± TGF-β1 (48 h; 10 ng/mL). Phosphorylated peptides were quantified and identified using MaxQuant software package. A web based Phospho-Analyst platform was used for statistical and gene ontology analysis. Benjamini-Hochjberg test (adjusted p value of 0.05) was used to determine significantly regulated phosphoproteins.
Results. 260 phosphosites mapping to 238 proteins were downregulated with NECA treatment compared to vehicle control, whereas 155 phosphosites (on 138 proteins) were upregulated. Following TGF-β1 treatment, NECA induced significant downregulation of 67 phosphosites mapped to 62 proteins whereas 35 phosphosites (on 35 different proteins) were upregulated compared to vehicle control. Gene ontology analysis showed overrepresentation of NECA regulated phosphoproteins in biological processes including actin-myosin structure organization and regulation of cytoskeleton in the absence and prescence of TGF-β1, respectively.
Discussion. A_{2B} receptor activation in human CFs regulates phosphoproteins involved in cytoskeleton and actin-myosin structure organisation. Future analysis will evaluate the subsequent influence on the modulation of fibrosis.

Vecchio EA *et al* (2016) Biochem Pharmacol 117:46-56.

P539. Characterisation of the pro-atherosclerotic orphan G protein-coupled receptor, GPR146

Brendan P Wilkins[1], Jack Zhang[1], Asuka Inoue[2], Marianne Martinello[3], Blake Cochran[1], Rowena Bull[3], Nicola J Smith[1]. School of Biomedical Sciences, UNSW Sydney[1], NSW, Australia; Graduate School of Pharmaceutical Sciences, Tohoku University[2], Sendai, Japan; The Kirby Institute, UNSW Sydney[3], NSW, Australia.

Introduction. GPR146 is an orphan G protein-coupled receptor that has a convincing pro-atherosclerotic role through upregulation of the cholesterol biosynthesis pathway. Inhibition of this receptor may be particularly useful with treatment-refractory familial hypercholesterolaemia. However, the molecular pharmacology of this receptor remains understudied. Proinsulin C-peptide and foetal bovine serum (FBS) are proposed activators of GPR146, although the pairing with C-peptide has not yet been reproduced by an independent research group and the active component in FBS has not yet been identified.
Aims. The aim of this study was to validate previously proposed ligands for GPR146.
Methods. C-peptide and FBS were tested using the following assays: reporter gene assays to investigate Gαs, Gαi/o, Gαq/11, and Gα12/13 signalling; a NanoBiT assay for β-arrestin recruitment; and Western blot or a BRET1-based biosensor for ERK1/2 phosphorylation (pERK1/2). A panel of 58 human sera was screened at GPR146 using Western blot probed for pERK1/2; the threshold for "hit" selection was set at ±2xSD. Human sera identified as "hits" were then further characterised using G protein- and arrestin-deficient HEK293A cells.
Results. Neither C-peptide nor FBS activated GPR146 in any assays tested (n=5); assay validity was confirmed by multiple positive controls. An overall increase in pERK1/2 was observed in response to human serum in GPR146-expressing cells compared to cells not expressing GPR146 ($P<0.0001$, paired t-test). 47/58 human serum samples elevated pERK1/2, with 5 surpassing the upper hit threshold indicating activation of GPR146.
Discussion. In this study, previously proposed ligands for GPR146 were not reproduced, indicating that C-peptide is not, and FBS does not contain, the endogenous ligand for GPR146. Instead, human serum was identified as an activator of GPR146. Future studies with human serum may identify the endogenous ligand for GPR146.

Yu et al. 2019. Cell. 179(6):1276-1288.e14.
Yosten et al. 2013. J Endocrinol. 11;218(2):B1-8.

P540. A novel single-cell fluorescence microscopy-based analysis for the detection of biomarkers of cellular senescence

Vijayraghavan Seshadri[1,2], Iman Azimi[1,2], School of Pharmacy and Pharmacology, University of Tasmania[1], Hobart, TAS, Australia; Department of Pharmacology, Monash University[2], Clayton, VIC, Australia.

Introduction. Cellular senescence is a complex state of irreversible cell cycle arrest with a secretory phenotype associated with age-related diseases. Senescence-associated beta galactosidase (SA-β gal) is one of the hallmark biomarkers of senescent cell detection along with the increased P53 and P16 expression, and cell and nucleus areas. Traditional method of SA-β gal determination employs cytochemical or histochemical staining and manual counting of positively stained cells. This method, however, has multiple disadvantages. In addition to its laborious procedure, these approaches often assess a population of cells as a whole. As a result, given the considerable heterogeneity among cells of a population in the level of different senescence markers, these analyses do not show a robust induction of senescence. This is particularly a disadvantage for developing drugs against senescence.
Aim. To develop a novel method of single-cell detection and analysis of senescence markers.
Methods. To establish an accelerated model of senescence, human-derived fibroblasts were treated with the chemotherapeutic agent, Mitomycin C (MMC) (50-600 nM), or vehicle for 72 h, and further cultured in normal media for five days. IN Cell Analyzer 2200 microscope was used to detect SA-β gal (stained using a commercial fluorescent enzymatic kit), and P21 and P16 proteins (stained by antibodies). IN Carta Image Analysis Software was used to quantify senescence markers. An "induction threshold" value was set by determining the value below which 90% of values in the control no-MMC group lie. Accordingly, percent of values above threshold was assigned for each group.
Results. Assessment of fluorescent intensity of SA-β gal, showed an around 2-fold increase in the average values of all cells with MMC treatment. We next plotted a histogram of values binned based on different SA-β gal fluorescence levels in cells. This histogram clearly indicated the considerable heterogeneity in SA-β gal levels. Using our "induction threshold" method, we showed an around 19-fold increase in SA-β gal levels in MMC-treated groups compared to the control. Similar trend of results was found in other biomarkers of cellular senescence including P53 and P16 expression, and cell and nucleus areas.
Discussion. We developed a novel analysis method of cellular senescence biomarkers at single-cell level. This method helps in identifying sub-populations of senescent cells and provides a robust platform for senotherapeutic discovery.

P565. Exploring Spexin-1 and Predicted Spliceoforms: Insights into GPR161 and GAL_2R Signalling

Kinjal J Patel[1], Simon R Foster[2], Alexander S Hauser[3], Nicola J Smith[1]. School of Biomedical Sciences, UNSW Sydney[1], Sydney, NSW, Australia; QIMR Berghofer Medical Research Institute[2], Brisbane, QLD, Australia; Department of Drug Design and Pharmacology, University of Copenhagen[3], Copenhagen, Denmark.

Introduction. In recent years, the use of *in silico* methods for the prediction of endogenous peptide ligands and their GPCRs has led to the identification of several novel pairings[1]. In one such study, the neuropeptide spexin-1 was proposed to be a potential ligand for the orphan GPCR, GPR161[1]. This neuropeptide has been shown in literature to bind galanin 2 (GAL_2) and 3 (GAL_3) receptors and has a physiological function that is seemingly reciprocal to their endogenous agonist, galanin[2]. Moreover, the precursor peptide for spexin-1 reveals several dibasic cleavage sites, indicating the possibility of multiple mature spexin spliceoforms.
Aims. To characterise the signalling profiles of spexin-1 and predicted spliceoforms at GPR161 and GAL_2.
Methods. Proximal interactions between GPCR and downstream effector molecules such as G alpha (Gα) proteins and β-arrestins, and downstream extracellular signal-regulated kinases (ERK) activation were quantified using bioluminescence resonance energy transfer (BRET) assays while changes in distal second messenger levels were measured using the cyclic AMP response element (CRE) or serum response element (SRE) reporter gene assays, upon ligand treatment. Appropriate positive controls were established for all assay systems in each individual experiment.
Results. Spexin-1 and its spliceoforms did not activate GPR161 through canonical G protein-dependent and -independent signalling pathways. Spexin-1 stimulated GAL_2, not only through G protein engagement, but also via β-arrestin2 recruitment. Several of the predicted spexin spliceoforms were found to be biologically active and stimulated GAL_2 in a G protein-dependent signalling assay.
Discussion. As the galanin receptor signalling axis is important for many physiological processes in the human body such as feeding, mood regulation and more, identification of novel agonists for these receptors may help uncover potential biased signalling mechanisms and ultimately improve therapeutics. Future experiments will aim to uncover differences in binding of spexin spliceoforms at GAL_2.
[1] Foster SR et al. (2019) Cell 179:895-908.e821
[2] Lv SY et al. (2019) Front Pharmacology 10:457

P541. A soft microenvironment uncouples TGF-β signaling from fibrogenesis in human pulmonary fibroblasts

Avanka Gunatilaka[1,2], Asres Berhan[1] Alastair Stewart[1,2]. [1]Department of Biochemistry and Pharmacology, [2]ARC Centre for Personalised Therapeutics Technologies, The University of Melbourne, Parkville, VIC, Australia

Introduction. Idiopathic Pulmonary Fibrosis (IPF) is a progressive disease in which the lung parenchyma undergoes irreversible remodelling. Activated myofibroblasts are notable drivers of the remodelling and fibrogenesis, and are further activated by both biomechanical and biochemical cues. We have demonstrated that in a permissively soft microenvironment, we are able to differentiate myofibroblasts to a lipofibroblast-like phenotype towards which TGF-β signalling is remodelled and appears 'afibrogenic'
Aims. We seek to characterize the extent to which lipogenic differentiation pathways and mechanosensitive fibrogenic transcription factors identified in the soft environment contribute to the afibrogenic phenotype.
Methods. Pulmonary fibroblasts were cultured either in a 2D stiff (conventional monolayer), 2D soft (hydrogels) or 3D soft (spheroid) microenvironment and the phenotypes engendered by these settings were characterized by a multi-omics approach, complemented with measurements of conventional fibrotic markers. Immunofluorescence staining was also performed to correlate the distribution of fibrogenic transcription factors with stimulation of lipogenic pathways (rosiglitazone) and microenvironment stiffness.
Results. Compared to fibroblasts cultured as a stiff 2D monolayer, fibroblasts cultured in the 3D soft microenvironment showed striking downregulation of cytoskeleton stress fibre protein and multiple subtypes of fibrillar collagen and connective tissue growth factor and upregulation of lipofibroblast markers such as perilipin 2. Moreover, the soft environment led to downregulation of fibrogenic transcription factors including Smad2/3 and YAP/TAZ. Notably, in spheroids TGF-β was not able to increase nuclear YAP/TAZ.
Discussion. The downregulation of fibrogenic transcription factors in the soft environment, and the apparent inhibition of their ability to localize in the nucleus in response to TGF-β serves as a likely explanation for the observed afibrogenic phenotype we have previously described. Ongoing work using hydrogels of tuneable stiffness will seek to establish a threshold stiffness to reveal the location of the stiffness 'cliff' beyond which some antifibrotic pathways may be compromised.

P542. Re-examining IRAK3 - an essential homeostatic checkpoint immune protein

Ilona Turek[1], H Trang Nguyen[1], Anna Axell[1], Bree Wright[1], Lubna A Freihat[2], David T Manallack[2], Tony Velkov[3], Helen R Irving[1]. La Trobe Institute for Molecular Science, La Trobe University[1], Bendigo, VIC, Australia; Monash Institute of Pharmaceutical Sciences, Monash University[2], Parkville, VIC, Australia; Department of Microbiology, Monash University[3], Clayton, VIC, Australia.

Introduction. IRAK3 (interleukin 1 associated receptor kinase 3) has a pivotal role in suppressing cytokine secretion (Kobayashi et al. 2002) that can also be dampened by increasing cellular cGMP levels (Nguyen et al. 2022). Bioinformatic searches reveal that IRAK3 contains a predicted crypto guanylate cyclase centre that generates cGMP (Freihat et al. 2019).
Aims. To examine how cGMP influences IRAK3 action in cells.
Methods. THP-1 wildtype and CRISPR/Cas 9 generated IRAK3$^{-/-}$ knockout cells were used in conjunction with cells containing NFκB reporter systems. cGMP, IL6 and TNFα were measured using ELISA kits.
Results. Subnanomolar levels of cGMP suppress NFκB activity and TNFα or IL-6 production in wildtype THP-1 monocytes stimulated by lipopolysaccharide (LPS) but not in IRAK3$^{-/-}$ knockout cells. Point mutations in the GC centre and surrounding residues of IRAK3 reduce its ability to generate cGMP. IRAK3$^{-/-}$ knockout cells transfected with wildtype IRAK3 regain their ability to suppress cytokine production following LPS treatment. IRAK3 constructs mutated in the death domain or the crypto guanylate cyclase centre fail to rescue IRAK3 function. However, membrane permeable cGMP can rescue the suppressive IRAK3 activity of mutants in the guanylate cyclase centre but not in the death domain.
Discussion. Surprisingly low levels of cGMP suppress cytokine production in the presence of IRAK3. These levels correlate with cGMP generated by IRAK3. We postulate that the protein scaffold of IRAK3 binds cGMP to create a cGMP enriched nanodomain enabling downstream signalling pathways to inhibit the inflammatory cascade.

Freihat L et al. (2019) Sci Rep 9: 15468
Kobayashi K et al. (2002) Cell 110:191-202
Nguyen HT et al. (2022) Int J Mol. Sci. 23: 2552

P543. Bombesin Receptor Subtype 3 as a potential target for lung adenocarcinoma

Mariah R Stavrou[1], Sara Ballouz[2], Nicola J Smith[1]. Dept of Pharmacol, School of Biomedical Sciences, UNSW Sydney[1], Kensington, NSW, Australia; School of Computer Science and Engineering, UNSW Sydney[2], Kensington, NSW, Australia.

Introduction. The Bombesin Receptor Subtype 3 (BB_3) is an orphan G protein-coupled receptor (GPCR) with no confirmed endogenous ligand. Bombesin receptors 1 (NMBR) and 2 (GRPR) are present in various adult tissues and are known to be overexpressed in many cancers. In contrast, BB_3 expression is largely restricted to foetal tissues, so the prospect that it is also overexpressed in cancer would make BB_3 a desirable cancer-specific target. Critical to the development of an orphan GPCR as a therapeutic target is the validation of its proposed pharmacology; for BB_3 this includes reports of constitutive activity and a proposed synthetic agonist.
Aims. To mine publicly available RNA-sequencing repositories and compare expression of BB_3 in healthy vs cancer tissue. Then, to validate recently described synthetic ligands and the pharmacological properties of BB_3.
Methods. Gene expression data was mined from healthy tissue control RNA-sequencing databases and cancer tissue databases. Sequencing counts were extracted as transcripts per million RNA reads (TPM) for BB_3 and various control genes. To measure constitutive and agonist activity at BB_3, HEK293 cells were co-transfected with BB_3 and luciferase reporter plasmids to measure downstream signalling of BB_3 with and without agonist stimulation.
Results. mRNA expression of BB_3 in healthy human tissue did not exceed 0.05 TPM at a bulk and single-cell level, which was negligible compared to physiologically active genes. However, BB_3 was expressed exclusively in lung cancer tissue. BB_3 activates Gs/Gq/G12 signalling in a ligand-independent manner, and couples to these same G proteins when stimulated with the synthetic agonist, MK5046.
Discussion. The exclusive expression of BB_3 in lung cancer tissue offers the possibility of selective and targeted treatment in a disease with high resistance to chemotherapy and other clinically used drugs. Given that a pharmacological 'toolkit' has been validated for the receptor, BB_3 can be further characterised in models of lung cancer despite its status as an orphan receptor and its lack of an identified endogenous ligand.

P544. Ebselen is unable to restore established cigarette smoke-induced neurocognitive dysfunction.

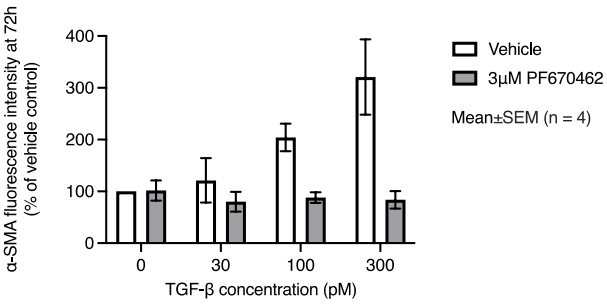
Alina Akhtar[1], Simone N. De Luca[1], Stanley Chan[1], Ross Vlahos[1].
[1]School of Health and Biomedical Sciences, RMIT University, Melbourne, VIC, Australia.

Introduction: Chronic obstructive pulmonary disease (COPD) is the third leading cause of death globally and becomes more taxing as extra-pulmonary conditions manifest. Memory and anxiety disorders have been reported in up to 60% of people with COPD compared to 12% of the general population. It has been proposed that lung inflammation and oxidative stress from exposure to cigarette smoke (CS) may "spill over" into the systemic circulation to promote the onset of these extra-pulmonary comorbidities.
Aim: To examine whether the anti-oxidant ebselen, when given after established lung disease (i.e., therapeutically), can improve lung inflammation and anxiety and memory impairments.
Methods: Male BALB/c mice were exposed to room air or to CS for 8 weeks. Following 8 weeks of CS exposure, mice were treated with ebselen (10 mg/kg) or vehicle (5% CM-cellulose) for 30 days alongside CS exposure. Mice then underwent neurocognitive testing (open field test and the novel object recognition [NOR] test [n=10-12]). Mice were then euthanised and lung (bronchoalveolar lavage fluid cellularity), systemic (TBARS), neuroinflammation (microglial morphology) and oxidative stress were assessed.
Results: CS-exposed mice treated with vehicle displayed increased BALF cellularity (p<0.0001) and ebselen reduced BALF neutrophilia (p<0.0001). This was associated with an attenuation in the expression of pro-inflammatory (*Il6, Tnf*) in CS ebselen mice to sham levels and a partial attenuation of oxidative stress (*Cybb*) gene expression. CS exposure increased systemic lipid peroxidation and was significantly improved by treatment with ebselen (p<0.05). Despite the improved pulmonary inflammatory profile, ebselen failed to improve the anxiety-like behaviour in the open field and working memory in the NOR test.
Discussion: CS exposure caused BALF inflammation and anxiety-like behaviour and working memory deficits like those seen in humans with COPD. Ebselen reduced CS-induced pulmonary inflammation and oxidative stress but failed to improve neurocognitive impairments. Future work will assess the mechanisms driving this neurocognitive decline.

P545. Casein kinase 1 delta/epsilon inhibition: a promising solution to unaddressed airway remodelling

Nguyen TH Tran[1], Daniel Tan[1], Alastair G Stewart[1,2]. Dept of Biochemistry and Pharmacology, Univ of Melbourne[1], Parkville, VIC, Australia; ARC Centre for Personalised Therapeutics Technologies[2], Parkville, VIC, Australia.

Introduction. Airway remodelling is a prominent feature underlying airway hyperresponsiveness (AHR) and is related to asthma severity. However, AHR is not effectively addressed by current asthma treatments. Transforming growth factor-beta (TGF-β) plays notable roles in remodelling by inducing cytokine production, fibrosis and thickening of the airway smooth muscle (ASM) layer. Inhibition of casein kinase 1 delta/ epsilon (CK1δ/ε), a recently identified downstream effector of TGF-β signalling, showed potential therapeutic actions in epithelial and fibroblast studies (Keenan et al, 2018).



Aims. To examine the impact of CK1δ/ε inhibition on TGF-β-induction of secretory and contractile properties of ASM.
Methods. Primary human ASM cultures were treated with the CK1δ/ε dual inhibitor PF670462 30 minutes prior to TGF-β1 stimulation. ELISA was used to measure levels of key fibrotic and inflammatory cytokines after 24h incubation. Alpha-smooth muscle actin (α-SMA) expression and its organisation into stress fibres after 1-3 days of drug treatment were assessed to elucidate contractile phenotype of the ASM cells using immunofluorescence.
Results. The marked release of IL-6, IL-11, PAI-1 and IGFBP-3 induced by TGF-β1 (100 pmol/L) was concentration-dependently inhibited by PF670462 (0.01-0.30 μmol/L), whereas higher concentrations were required (1-3 μmol/L) to prevent TGF-β1-induced α-SMA expression and stress fibre organisation.
Discussion. TGF-β is a strong promotor of pro-inflammatory and fibrogenic microenvironment and induces a hypercontractile phenotype of ASM cells. This study further supports the therapeutic potential of targeting CK1δ/ε to address remodelling and hyperresponsiveness, particularly in steroid-insensitive severe asthma.
[1]Keenan CR et al (2018) Front Pharmacol 9:738.

P546. Human osteoclast differentiation after induction by mRNA of NFATc1

Sirada Srihirun[1], Chareerut Phruksaniyom[1], Pornpun Vivithanaporn[2], Kran Suknuntha[2].
Department of Pharmacology, Faculty of Dentistry, Mahidol University[1], Bangkok, Thailand; Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University[2], Samut Prakan, Thailand.

Introduction. The inhibition of osteoclast, a bone-resorptive cell, is a key model for treating diseases associated with bone loss. Although in vitro human osteoclasts can be generated from RANKL-stimulation of peripheral mononuclear cells (PBMCs), the osteoclast differentiation rate is low with time-consuming. Because NFATc1 is a master transcription factor downstream of the activation of the RANK receptor, we hypothesized that the expression of NFATc1 by transfection with modified mRNA may enhance osteoclast differentiation efficacy with a shorter period.
Aims. This study aims to generate osteoclasts from human PBMCs by induction with modified mRNA of NFATc1.
Methods. Human PBMCs were isolated from the venous blood of healthy donors (n=3) by the Histopaque density gradient technique. Human macrophages were expanded and separated from PBMCs by supplemented with 25 ng/mL M-CSF for 3 days. Modified mRNA of NFATc1 (10-20 ng) was transfected in human macrophage by Lipofectamine Messengermax. Osteoclast-specific markers, including TRAP-positive multinucleated cells and F-actin ring formation were determined.
Results. A day after transfection with 10 and 20 ng of modified RNA, NFATc1 was expressed in human macrophages at 3 and 13 folds, respectively. TRAP-positive multinucleated cells and F-actin ring were generated after transfection with 10 ng mRNA of NFATc1 for 3 days.
Discussion. Osteoclast formation can be induced by the expression of mRNA NFATc1 with a shorter period than conventional RANKL stimulation. However, the bone-resorptive function of osteoclast still needs further investigation.

P547. Involuntary exercise did not improve cigarette smoke-induced anxiety-like behaviour in male mice.

Rana Alateeq[1], Simone N. De Luca[1], Huei Jiunn Seow[1], Suleman Almerdasi[1], Stanley M.H. Chan[1], Wei Wang[1], Ross Vlahos[1].
[1] School of Health and Biomedical Sciences, RMIT University, Melbourne, VIC, Australia.

Introduction. Chronic obstructive pulmonary disease (COPD) is the 3rd leading cause of death globally leading to substantial economic and healthcare burden. People with COPD suffer from comorbid anxiety disorders which adversely impact their quality of life. Pulmonary rehabilitation, a pivotal therapeutic approach, has the potential to ameliorate dyspnoea and elevate the overall quality of life. A core component of this rehabilitation is aerobic exercise (AE) training. Literature has illustrated the positive impact of AE on alleviating anxiety symptoms; however, a subset of studies presents a contrasting viewpoint by indicating limited enhancements. Thus, it is imperative to delve into the intricate mechanisms that underlie this variability in patient response. We hypothesise that AE increases antioxidant and anti-inflammatory activity and will ameliorate cigarette smoke (CS)-induced anxiety-like behaviour and oxidative stress in mice.
Aims. To examine whether AE can improve lung and systemic inflammation and anxiety-like behaviour in CS-exposed mice.
Methods. Male BALB/c mice were exposed to either room air (sham) or CS from 9 cigarettes/day alongside involuntary treadmill exercise (50% of maximal speed) for 30 min/day for 8 weeks. After 7 weeks of this regimen, behavioural tests were conducted to assess anxiety-like behaviour. Bronchoalveolar lavage (BALF) and blood samples were collected to assess inflammation and oxidative stress. Brains were collected to assess microglial profiles.
Results. CS exposure increased BALF cellularity which was not altered by AE (p<0.05). Involuntary AE reduced CS-induced neutrophilia (p=0.04, n=16/group) and systemic lipid peroxidation (p=0.01, n=8/group) in the blood. Involuntary AE did not improve CS-exposed anxiety-like behaviours and impairments in social recognition memory (n=8/group). The anxiety-like behaviour in CS-exposed mice was not associated with an increase in serum corticosterone.
Discussion. CS exposure caused BALF inflammation and anxiety-like behaviour and AE was unable to prevent the anxiety-like behaviour. Surprisingly, serum corticosterone level was not affected by involuntary AE. AE reduced systemic oxidative stress, therefore future work will assess brain oxidative stress and microglial profile in key brain regions involved in anxiety.

P548. Pulmonary and vascular consequences of influenza A virus (IAV) infection in atherosclerotic mice

Madison Coward-Smith*[1], Stella Liong[1], Hao Wang[1], Steven Bozinovski[1], Antony Vinh[2] Felicia Liong[1], Doug A. Brooks[3], John J. O'Leary[4], Gemma Trollope[1], Mark Miles[1] and Stavros Selemidis[1]. [1]Centre for Respiratory Science and Health, School of Health, and Biomedical Sciences, RMIT University, VIC, 3083, Australia. [2]Centre for Cardiovascular Biology and Disease Research (CCBDR), La Trobe University, Bundoora, VIC, 3083, Australia. [3]Clinical and Health Sciences, University of South Australia, Adelaide, 5001, Australia. [4]Discipline of Histopathology, School of Medicine, Trinity Translational Medicine Institute (TTMI), Trinity College Dublin, Ireland.

Introduction. Influenza in people with atherosclerosis increases their risk of plaque destabilization, myocardial infarction, and death *via* largely unknown mechanisms.
Aim. To determine the effect of IAV infection on lung and vascular function and atherosclerosis progression in mice.
Methods. 5-wk old APOE$^{-/-}$ mice were placed on a high fat diet for 7 weeks. At the beginning of week 8, mice were intranasally inoculated with a low dose of the mouse adapted Hk-x31 strain of IAV to recapitulate a mild seasonal form of influenza disease. The degree of atherosclerosis was assessed at week 14 with oil-red O staining. Blood pressure and pulse rate were taken weekly. Myography was used to assess endothelial-dependent (Ach; 10^{-9}-10^{-5}M) and independent relaxation (sodium nitroprusside 10^{-5}M), respectively. Lung function and the mean linear intercept (MLI) to determine alveolar enlargement were assessed. The Fulton index was measured for right ventricular hypertrophy.
Results. Aorta from IAV infected mice failed to relax to both ACh and SNP and displayed an impaired contraction to the vasoconstrictor, U46619 indicating a substantial reduction in endothelial and smooth muscle function. Infected APOE$^{-/-}$ mice exhibited significantly reduced plaque coverage when compared to uninfected controls. Infected mice displayed significantly decreased FEV0.1/FVC ratios when compared to controls, and this was associated with right ventricular hypertrophy and significantly higher MLI. IAV also caused significant systolic hypotension and bradycardia during the acute phase of the infection.
Discussion. IAV infection in APOE$^{-/-}$ mice causes profound dysfunction of the aorta and pulmonary remodeling manifested as damage to the alveoli and lung dysfunction. IAV also caused bradycardia and hypotension and right ventricular hypertrophy, suggestive of pulmonary hypertension.

P549. Investigating the application of an in vitro 3D cell culture technique to improve the translational potential of novel immunotherapy drug treatment strategies for pleural mesothelioma.

Aimee Stenekes[1,2], Chantal Donovan[2], Helen Ke[1], Ling Zhuang[1], Huaikai Shi[1], Ben Johnson[1]. Asbestos and Dust Diseases Research Institute[1], Sydney, NSW, Australia; School of Life Sciences, University of Technology Sydney[2], Sydney, NSW, Australia.

Introduction. Pleural mesothelioma (PM) is a rare and aggressive cancer that develops in the mesothelial lining of the lung pleura following exposure to asbestos. Despite the recent availability of immunotherapy treatments, the median survival rate of PM patients is only 18 months, highlighting the urgent need for improved immunotherapy treatment strategies. There have been minimal advancements to current standard care due to inconsistencies between preclinical 2D model and clinical trial drug screening data. However, 3D cell culture systems that more accurately recapitulate the complex structure and microenvironment of mesothelioma tumours make them ideal models for pre-clinical screening of novel immunotherapy drugs.
Aims. To develop a novel 3D co-culture model of mesothelioma using HLA-matched patient-derived tumour cells and healthy volunteer-derived B&T cells to facilitate prospective preclinical immunotherapy drug screening.
Methods. Mesothelioma cell lines were HLA-matched with B&T cells isolated from healthy volunteer peripheral blood samples. Matched samples were then co-cultured on novel decellularized porcine lung scaffolds. Formalin-fixed scaffold sections were stained with clinically validated mesothelioma biomarkers (BAP1, CDK2NA, p53) and B&T cell markers (CD3, CD80) via histological assessment through immunohistochemistry (IHC) to confirm mesothelioma and immune cell co-existence.
Results. Four HLA-matches to two mesothelioma cell lines were obtained from 30 healthy volunteer blood samples. Cells were able to grow on and penetrate the porcine scaffold for up to 14 days. IHC staining of CD3 and CD80 cells confirmed the presence of B&T cells in our novel 3D model. Presence of cancer cells was also confirmed through the assessment of clinically validated mesothelioma biomarkers, including loss of BAP1 and CDK2NA expression, and the presence of p53.
Discussion. Our study successfully established a novel 3D scaffold cell culture model of mesothelioma that facilitates the co-existence of mesothelioma and immune cells. We anticipate this 3D mesothelioma model can be utilised for prospective preclinical immunotherapy drug screening studies.

P550. Development of a Precision Medicine Competency Framework for the Therapeutics Industry

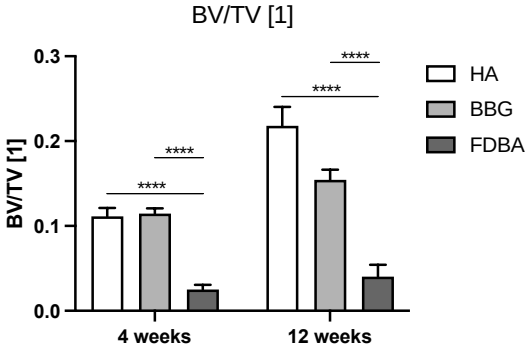
Orin Chisholm[1] and Nicky Conway[1,2]. Sydney Pharmacy School[1], University of Sydney, Sydney, NSW, Australia. GenomePlus Pty Ltd, Sydney, NSW, Australia

Introduction: The successful adoption of precision medicine relies on the development of effective treatments and judicious utilization within a supportive healthcare system. Embracing a learning healthcare capability will be crucial to navigating the disruptions arising from rapid scientific and technological innovation.
Aim: to build a Precision Medicine (PM) competency framework that can be used across the Medical Technology and Pharmaceutical (MTP) industries to build a confident and capable workforce, support cross-disciplinary work and collaboration, and instil a continuous learning mindset.
Methods: A desktop research review of current literature, curriculum, and healthcare trends identified a core set of domains and subdomains related to precision medicine competencies. A survey was distributed to the Industry Genomics Network Alliance (InGENA) members to confirm the relevance and applicability of the domains and subdomains to their current work practice and their expected work practice in 5 years' time.
Results: Four domains were identified: medical science and technology; translational and clinical application; governance and regulation and professional practice. Each domain has a series of subdomains and patient needs were integrated across all four of the domains. Survey results confirmed the applicability of these domains to the MTP industry.
Discussion: The Framework was well accepted by industry, with a strong interest from related disciplines including allied health professionals. Given the pace of change this framework will need regular review and updating.

P551. Bone regeneration potential of 3D printed hydroxyapatite in a rat model

Ticomporn Luangwattanawilai[1], Faungchat Thammarakcharoen[2], Autcharaporn Srion[2], Kasem Rattanapinyopituk[3], Jintamai Suwanprateeb[2], Ruedee Hemstapat[1]. Dept of Pharmacol, FCs of Sci, Mahidol Univ[1], BKK, Thailand; MTEC, NSTDA[2], PTH, Thailand; Dept of Vet Pathol, FCs of Vet Sci, Chulalongkorn Univ[3], BKK, Thailand.

Introduction. Hydroxyapatite (HA), an alloplastic material that has been widely used as scaffold for bone tissue engineering owing to its osteoconductivity and biocompatibility.
Aims. This study was aimed to compare the *in vivo* bone regeneration potential of newly invented HA (Suwanprateeb et al, 2010) prepared by powder based three-dimensional printing (3DP) in couple with a low temperature phase transformation technique to that of commercial bone substitute materials including bovine bone graft (BBG) and freeze-dried bone allograft (FDBA) in rat calvarial defect model.



Methods. The bone regeneration potential of HA was compared to BBG and FDBA in a rat calvarial full thickness defect model. General anaesthesia was administered by inhalation of 3-5% isoflurane. New bone formation was investigated with micro-computed tomography (micro-CT), histology and immunohistochemistry at 4-and-12 weeks post-implantation.
Results. Implantation with HA and BBG scaffolds showed a significant increase in new bone formation within bone defect site compared to FDBA at both 4-and-12 weeks post-implantation. Moreover, Osteocalcin immunohistochemistry result revealed a significant increase in number of osteoblasts and osteocytes in HA group compared to FDBA group at 12 weeks post-implantation.
Discussion. 3D printed HA could facilitate new bone formation and induce bone cells activity greater than FDBA, while no significant difference between HA and BBG was observed.

J. Suwanprateeb et al (2010) J Mater Sci Mater Med 21:419-429

P552. New methods for designing patient-centred clinical trials for epilepsy and cannabidiol drugs.

Linda Truong [1], John A Lawson [1,2]. Jennifer H Martin. School of Medicine and Public Health, University of Newcastle[1], Newcastle, NSW, Australia; Office of Health and Medical Research, NSW Ministry of Health [2], Sydney, NSW, Australia.

Introduction. Current clinical trials of epilepsy usually target reduction in seizures[3] and do not commonly involve patient participation or perspectives. We believe the development of patient-centric clinical trial models for epilepsy with patient-reported outcomes would be helpful to inform national clinical practice and policy decisions [1,2]. Aim. We aimed to develop a study that collects end-user insights on how to improve the design of clinical trials for epilepsy through the creation of advisory groups and focusing on patient experiences. For the initial study, we were interested in experiences of using cannabidiol. Methods. After ethics approval (2022/ETH01648), we planned to use qualitative research methodologies such as surveys, questionnaires and recorded semi-structured interviews to understand the perceptions, and experiences of epilepsy clinical trials and cannabidiol medicines. Audio-recorded interviews will be transcribed verbatim and analysed using thematic analysis using NVivo to identify emerging themes[4]. We planned a sample size of 40 using the concept of 'information power'. Further, the qualitative data collected will be analysed to develop a patient-acceptable epilepsy clinical trial protocol for the implementation of scalable Phase 2 clinical trials using cannabidiol. Results. To date, we have developed advisory groups, formed through either an Expression of Interest (EOI) process or by direct invitation. These advisory groups consist of patients with lived experience of epilepsy, clinicians, professionals with leadership roles in clinical trials in epilepsy, or representatives of the pharmaceutical clinical trials sector. Discussion. Patient participation in the design of clinical trials for epilepsy is a priority, mainly because previous studies on issues in the design of the protocol have meant that the results are not always widely applicable to the patient or the general epilepsy population. We expect this feedback to improve the precision, validity, and patient-relevant clinical outcomes for testing cannabidiol in epilepsy, and thus a model that is helpful for the development of all new therapeutics.

[1] NHMRC, (2016) NHMRC Statement. [2] FDA (2022) Food and Drugs Administration (FDA) Guidelines. [3] European Medicines Agency (2010). EMA Guidelines. [4] Braun, V et al. (2019) Qualitative Research in Sport, Exercise and Health. 11:589-597

P553. What is a Non-Significant Risk Device Clinical Study?

Melissa Dworak[1], Ingrid Leth[2], Michael Strauss[2], Felicity Grzemski[1], Jeanne Novak[2]. Novotech Drug Development Consulting-Novotech-CRO[1], Melbourne, VIC, Australia; Novotech Drug Development Consulting-Novotech-CRO[2], Boulder, CO, USA.

Introduction: Medical device clinical trials (MDCTs) are investigations or examinations undertaken to assess the safety or the performance of a medical device in terms of its use in either the treatment, prevention or diagnosis of diseases in human subjects. The process to get a medical device into a clinical trial is determined by the risk posed by the device. There are multiple pathways to achieve market entry of medical devices and In-Vitro Diagnostics (IVD) based on the device risk profile. One pathway not always known or considered by Sponsors is the Non-Significant Risk (NSR) study path. The value of conducting Non-Significant Risk (NSR) studies that only require approval of an IRB or ethics committee can significantly accelerate the clinical study plan and acquisition of data.
Aims: The intention of this abstract is to describe the requirements of this accelerated pathway into clinical studies, and the types of devices for which this pathway may be utilized.
Discussion: In different regulatory jurisdictions, the potential regulatory risk of a device can change the review process for gaining approval to conduct a clinical trial, in cases where clinical safety/efficacy data is needed. Choosing the right pathway in the early phase of device development can save time and reduce cost. To qualify as a non-significant risk device, the device must not pose a serious risk to human subjects. Of note, the risk determination of a device is based on the proposed use of the device in an investigation not the device itself. The potential harm the device could cause as well as the potential harm of any procedure associated with the device should be considered. Once the risk of a device had been identified, the pathway to get clinical trial approval can be confirmed. In the US the FDA is involved in approval of significant risk device clinical trial or an investigational device exemption (IDE) approval. However, a non-significant risk device clinical trial can be approved by the Institutional Review Board (IRB) at the site where the study is to be conducted. In Australia the TGA is currently considering changes whereby high risk (invasive or implantable) devices are approved through the CTA pathway (TGA review and approval) and low risk devices can be approved through the CTN pathway (HREC review and approval).
Conclusion: For simple low risk devices that still require clinical studies, as well as IVDs, NSR studies provide an accelerated pathway to entering clinical studies.

P554. Nonclinical and Manufacturing Considerations for Novel Live Biotherapeutic Products

Sharleen Menezes[1], Christopher Hartnett[2], Felicity Grzemski[3], Jeanne Novak[2]. Novotech Drug Development Consulting, Novotech-CRO, Sydney, NSW, Australia[1]; Boulder, CO, USA[2]; Melbourne, VIC, Australia[3].

Introduction. A live biotherapeutic product (LBP) contains a single species or consortium of human commensals isolated from healthy human donors with the aim for use in the prevention, treatment, or cure of a disease. There has been a major effort in developing LBPs for indications including obesity, gastrointestinal (e.g., ulcerative colitis) neurodegenerative (e.g., Alzheimer's Disease), and as adjunct cancer (e.g., checkpoint inhibitors) therapies. Due to the diversity of LBP substrate and programs, it has become clear that the preclinical development and evaluation of LBPs requires a case-by-case approach.
Aim. The aim of this abstract is to highlight some considerations learned from our experiences in nonclinical and manufacturing development of LBPs cumulating in successful clinical trial entry.
Discussion. The preclinical evaluation of LBPs requires nonclinical, chemistry, manufacturing, and controls (CMC) and regulatory strategies to support a proposed clinical plan. LBPs have been shown to be efficacious in disease treatment, but in many cases, the mechanism of action (MOA) is unknown. The MOA may be multifactorial and include activation of cell pathways, modulation of the mucosal immune system or influencing existing microorganism:host interactions. A demonstration of preclinical efficacy can prove challenging due to the diversity of the human microbiome and its ability to be mimicked in an animal species. Initial study consideration should be taken in terms of safety, including Generally Recognised As Safe (GRAS) status, or listing on the Qualified Presumption Safety list for its use in foods. Also, while traditional toxicology studies may not be required, a translocation study is recommended for LBPs. The CMC product development requires consideration of large-scale culture of strict anaerobes, drying processes to maintain organisms in a vegetative state, and formulation strategies necessary to transit the gastric environment. Endpoints from nonclinical studies for justification of clinical dose require CMC considerations including potency measurement (viable cells), and potential for engraftment. The FDA has updated the guidance document for LBP development and collectively, global regulatory authorities now recognise LBPs within their own category (separate from probiotics).
Conclusion. The preclinical development of LBPs requires a multifaceted strategic approach to ensure quality, efficacy, and safety to support clinical programs.

P555. Automated In-Vitro Population Bioequivalence for Inhaled Products to Meet the FDA Guidance on Budesonide Using Phoenix® WinNonlin®

Chandramouli Radhakrishnan[1], Ana Henry[1], Jean-Michel Cardot[2] Certara[1], NJ, USA; SAS Borvo[2], Ceyrat, France.

Introduction. Oral inhaled and nasal drug products are extensively used in dosage forms. Population bioequivalence methods are recommended by the US Food and Drug Administration for in vitro in Vivo bioequivalence assessment (FDA Guidance, 2001,2003,2012). The population Bioequivalence approach compares the particle and agglomerate Particle Size Distribution in the nebulized aerosol between the test and reference. Phoenix® WinNonlin templates can guarantee standardization and accuracy of the calculations and statistical analysis needed to show population bioequivalence for in-vitro inhaled products.
Methods. Phoenix templates were developed to meet the specific recommendations outlined in the FDA guidance on budesonide as recommended by FDA guidelines. The guidance also recommends the particle size measured at different life stages (beginning, middle, and end) of the container, and the Phoenix templates can handle these data.
Results. PBE analysis of budesonide was performed using Phoenix WinNonlin workflows, using datasets provided by the FDA in their guidance. Critical results obtained with Phoenix matched results reported by FDA.
Discussion. Phoenix templates can be easily reused with different datasets. The Phoenix templates and example projects presented in this poster are available for download at Certara University.
Reference:
FDA. Guidance for industry. Statistical approaches to establishing bioequivalence. 2001. http://www.fda.gov/downloads/Drugs/Guidances/ucm070244.pdf.
FDA. Draft guidance for the industry. Bioavailability and bioequivalence studies for nasal aerosols and nasal sprays for local action. 2003. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070111.pdf.
FDA. Draft guidance on budesonide. 2012. https://www.accessdata.fda.gov/drugsatfda_docs/psg/Budesonide_Inhalation_Sus_20929_RC_09-12.pdf

P556. Nonclinical and CMC Considerations for the Development of siRNA Therapeutics

Sweta Kumar[1], Sharleen Menezes[2], Melisa Anggraeni[3], Felicity Grzemski[4], Jeanne Novak[5]. Novotech Drug Development Consulting-Novotech-CRO, Bangalore, Karnataka, India[1]; Sydney, NSW, Australia[2]; Brisbane, Qld, Australia[3], Melbourne Vic, Australia[4]; Boulder, CO, USA[5].

Introduction: siRNAs are short, noncoding, double-stranded RNA that can suppress gene expression by targeting and degrading mRNA through an RNA-induced silencing complex. They offer many advantages, such as targeting diseases that are not always treatable with the use of small molecules or proteins as they can specifically target a wide range of genes. However, despite showing promise *in vitro*, siRNAs face numerous limitations *in vivo* such as its elimination, immune destruction, instability, toxicity, and off-target effects.
Aim: To highlight some of our experiences in the nonclinical and CMC development of siRNA therapeutics.
Discussion: Recently, advances in RNA interference technology have addressed stability and specificity issues and led to targeted delivery, and improved stability and pharmacokinetic properties. However, safety concerns, quality and dose-limiting toxicities remain among the reasons for the failure of siRNA therapies. Currently, no official regulatory guidance specific to siRNAs are available, requiring reliance on a hybrid of small molecule and biologics guidances for nonclinical and CMC development. Hence, early engagement with the relevant regulatory bodies is essential to determine product specific development needs. CMC challenges include requirement of development stage appropriate CMC information, applicability of USP salt policy, impurity identification, characterisation, and qualification thresholds. Nonclinical studies should be fit-for-purpose and include consideration of the therapeutic RNA construct, the carrier/delivery system, individual components of the carrier, and the combined final product. The toxicological outcomes related to the delivery platforms and their species-specific nature should also be included. For example, hepatotoxicity as an off-target effect was observed in rats with all GalNAc-siRNA conjugates. Also, owing to the lack of sequence homology between humans and rodents, the use of surrogate siRNA sequences may be considered to de-risk the potential for pharmacology-related on-target toxicities.
Conclusion: Overall, siRNA therapeutics development is evolving and holds extreme promise. When supported by the well-designed predictive nonclinical studies with insight into the effect of disease complications on biodistribution and toxicity of siRNA drugs, they can move successfully into and through clinical studies.

P557. Oxyresveratrol and resveratrol attenuate cadmium-induced cytokine release in human astrocytes

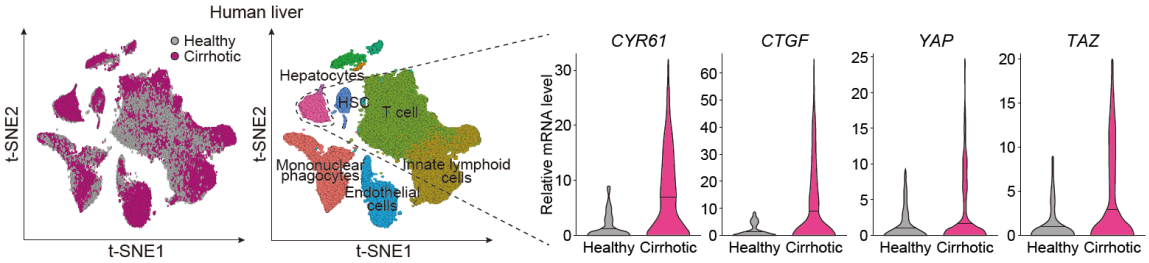
Vivithanaporn P[1]*, Settacomkul R[1], Woonfak P[1], Phuagkhaopong S[2]
Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University[1], Samut Prakan, Thailand; Department of Pharmacology, Faculty of Medicine, Chulalongkorn University[2], Bangkok, Thailand.

Introduction. The inflammatory responses under cadmium exposure could play an important role in the development of glioblastoma and neurodegenerative diseases. Upregulation of inflammatory mediators, specifically IL-6, IL-8, and CCL-2, is associated with a poor prognosis in patients with glioblastoma multiforme. Resveratrol and oxyresveratrol are natural polyphenolic compounds possessing antioxidant and anti-inflammatory properties in astrocytes and microglia.
Aim. We aimed to investigate the capability of resveratrol and oxyresveratrol to inhibit the inflammation in cadmium-induced astrocytes and explore mechanisms underlying their inhibitory effects.
Methods. Human astrocytoma cells (U-87 MG) were stimulated by cadmium with or without either resveratrol or oxyresveratrol. Cell viability is measured by MTT assays. The mRNA expression and release of IL-6, IL-8 and CCL2 are measured by real-time PCR and ELISA. The activation of MAPK pathways was determined by detecting phosphorylation of ERK1/2, JNK and p38 using Western blotting.
Results. Resveratrol at 100 μM or less showed no cytotoxicity to cells, while oxyresveratrol at the same concentration reduce cell viability by 50%. Co-treatment of human astrocytes with resveratrol and oxyresveratrol at concentrations of 25 and 50 μM together with the exposure to cadmium (10 μM) considerably diminished pro-inflammatory cytokines (IL-6, IL-8, and CCL-2) in both gene expression and protein secretion levels compared with those of the cells treated with cadmium alone; resveratrol was more potent than oxyresveratrol. Furthermore, resveratrol and oxyresveratrol decreased the phosphorylated levels of ERK1/2, JNK, and p38, proteins in a similar pattern.
MEHP induced ER stress-associated mRNAs, including PERK, ATF-4, CHOP, ATF6, IRE1α, spliced XBP-1 and CHOP. The increase of CHOP and spliced XBP-1 protein expression are found in MEHP-treated cells.
Discussion. The potent anti-inflammatory properties as well as the lower cytotoxicity suggest resveratrol as a potential pharmacologic agent to protect astrocytes following cadmium exposure.

P558. Stress-driven suppression of the Hippo pathway accelerates liver injury

Ari Kwon[1], Na Young Lee[1], Jae-Hyun Yu[1], Myeung Gi Choi[1], Ja Hyun Koo[1]. Coll of Pharm, Seoul Natl Univ[1], Seoul, Republic of Korea.

Introduction. In the liver, hepatocytes are the primary target for acetaminophen (APAP) intoxication, leading to transmission of signals to the nucleus and activation of a transcriptional program. However, how cellular stress affects the nuclear transcription machinery during liver injury remain unclear.
Aims. The molecular interplay between cellular stress and YAP/TAZ in the liver is investigated.
Methods. YAP/TAZ activity was analyzed in hepatocytes from patients with cirrhosis using single-cell transcriptomic data and in mice with APAP intoxication. The impact on Hippo pathway regulation following acetaminophen and CCl4 intoxication was analyzed using RNA sequencing. Phos-tag immunoblotting, immunocytochemistry, and qRT-PCR were used to assess YAP activity. CRISPR-mediated knockout or knock-in cells were used for mechanistic studies. Mice with hepatocyte-specific disruption of Yap and Taz were used to examine their role in liver injury.
Results. YAP activity was substantially enhanced in hepatocytes following liver injury; it was associated with cellular stress markers and inflammation in humans and mice. Cellular stress from different mechanisms robustly promoted YAP/TAZ dephosphorylation, nuclear accumulation, and target gene transcription. Acute liver injury was suppressed in hepatocyte-specific Yap/Taz knockout mice.
Discussion. Cellular stress is tightly connected to YAP/TAZ activity in hepatocytes. YAP/TAZ are required for transcriptomic changes following hepatocyte stress during liver injury.



P559. Impact of target expression on target safety assessment of ADCs

Phumzile Sikakana[1], William Humfrey[1], Michelle Blissett[1], Nicholas Coltman[1], Sean Hammond[1], Helen Garside[1], James Sidaway[1], Ruth Roberts[1]. ApconiX, Alderley Park, Alderley Edge, United Kingdom.

Introduction. Target safety assessments (TSAs) use target biology, gene and protein expression, humans and animal genetics and drug safety data to understand the potential safety risks associated with modulating a drug target. TSAs are used within drug projects to identify and mitigate risks to help with informed decision making.
Aims. To guide TSAs by 1) determining if target expression data for antibody-drug conjugates (ADCs) correlates with reported clinical toxicity, and 2) assessing the expression of ADC targets in cancer cell lines versus normal human cells.
Methods. Using publicly available data from drug labels for twelve regulatory approved oncology ADCs, clinical adverse events (AEs) were collated. The twelve ADCs included in our study covered ten individual targets. Data on target mRNA and protein expression in normal tissues was obtained from datasets deposited by the Human Protein Atlas (HPA) consortium, categorized as high, medium, low or negligible expression across key organ systems. HPA data was also used to assess the mRNA expression of each target in cancer cell lines relative to that in normal cells.
Results. Across all 12 ADCs, AEs occurred in 70% of organ systems that had high expression of the target and in 43% or organ systems where there was medium target expression. Half of the instances of high target expression occurred in the hematologic and immune organ system. However, all twelve ADCs had hematologic and immune AEs regardless of target expression level, suggesting a strong contribution of non-target related toxicity. Single cell mRNA data demonstrated that, within medium and high expressing tissues, the expression of most targets was greater (≥ 2-fold) in tissues with clinical AEs compared with those without. However, potential target-related clinical AEs were present even when there was a much higher expression of the target in a relevant cancer cell line compared with the average expression in affected tissues.
Discussion. Overall, as expected, commonly occurring clinically identified AEs were more frequent in organ systems where there was high expression of the antibody target. When considering TSAs for new ADCs, expression of the antibody target in normal tissues should be highlighted as a potential risk and an assessment made of the target expression in cancer cell lines versus normal cells. However, careful experimental assessment will be required to determine if high levels of target expression translate into clinical and/or nonclinical adverse effects.

P560. Computational models of retinoic acid receptor antagonists acting as endocrine disrupting chemicals

Raymond Lui, Slade Matthews. Computational Pharmacology and Toxicology Laboratory, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia.

Introduction. The retinoid signalling pathway is currently being advocated for inclusion in OECD endocrine disruptor testing guidelines due to its key roles in the hormonal regulation of development and reproduction, as well as crosstalk with other endocrine pathways. Accordingly, there is regulatory demand for quantitative structure – activity relationship (QSAR) models that enable high-throughput screening of retinoid-based endocrine disrupting chemicals. In particular, chemicals with antagonistic activity at the retinoic acid receptor (RAR), the main nuclear receptor of the retinoid pathway, have been identified as potential disruptors of retinoic acid signalling in spermatogenesis leading to a period of male infertility.
Aims. To develop computational QSAR models that classify chemicals as active or inactive RAR antagonists. Specifically, we compare the prediction accuracy and mechanistic interpretability of models derived from various *in silico* molecular representations ranging in scale and complexity.
Methods. Ligand-based Morgan topological fingerprints and Mordred constitutional descriptors were calculated from the Tox21 RAR antagonist dataset. Automated machine learning was used to model QSARs between the molecular features and Tox21 experimental results. Permutation feature importance was performed to identify the molecular features the machine learning models deemed most relevant to predicting RAR antagonism.
Results. The fingerprint model predicted with higher sensitivity and balanced accuracy, suggesting RAR antagonistic activity is strongly governed by specific substructures in retinoid-based endocrine disrupting chemicals. Indeed, fingerprint bits identified as important by the machine learning model were consistent with structural moieties present in BMS-189453, a potent synthetic RAR antagonist with demonstrated antispermatogenic effects.
Discussion. Continuing work includes the exploration of structured-based representations that encode specific protein – ligand interactions within the RAR active site that permit deeper mechanistic considerations. The resulting ensemble of computational RAR antagonist models from this study can be practically deployed to address ongoing scoping efforts for the inclusion of the retinoid pathway in endocrine disruptor testing.

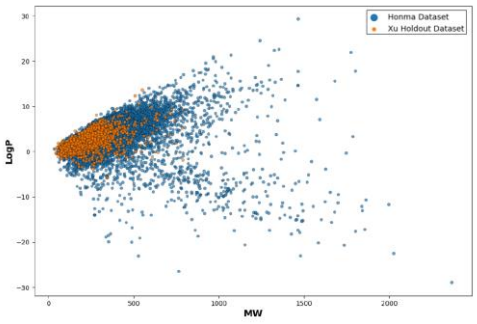
P561. MEHP-induced apoptosis, autophagy, and endoplasmic reticulum stress in human neurons and astrocytes

Sukprasert R[1], Suknuntha K[2], Setthakomkul R[2], Siangcham T[1], Vivithanaporn P[2]. Faculty of Allied Health Sciences, Burapha University[1], Chon Buri, TH; Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University[2], Samut Prakan, TH.

Introduction. Di(2-ethylhexyl) phthalate (DEHP) is widely used in food packaging and contaminated in water. It's an environmental endocrine disruptor that causes damage to the nervous system in rats. DEHP is metabolized into mono (2-ethylhexyl) phthalate (MEHP) by cytochrome P450 enzymes. DEHP induces apoptosis and endoplasmic reticulum stress in human SH-SY5Y cells and increases caspase 3 expression in mouse astrocytes. MEHP induces apoptosis in liver cells but there is no report of toxicity in neurons and astrocytes.
Aim. We compared the cytotoxic effects of DEHP and MEHP in human neurons and astrocytes and determined the underlying mechanisms.
Methods. The toxicity of the DEHP and MEHP on cell viability of U-87 MG and SK-N-SH cells by MTT assays at 24h. The apoptosis was measured using Annexin V FITC and 7-AAD staining. The formation of autophagic vesicles is determined by the Autophagic flux kit (Enzo Life Sciences, USA). The expression of mRNA and proteins related to apoptosis, autophagy, and ER stress were measured using real-time PCR and western blot analysis.
Results. The IC50 values of MEHP were 440.6 $\pm$ 0.96 µM and 281.9 $\pm$ 0.89 µM in SK-N-SH and U-87 MG cells, respectively. In contrast, DEHP up to µM showed minimal toxicity. The percentage of apoptotic SK-N-SH cells after exposed to 500 µM MEHP for 24h was 24.60% compared with 8.59% of DEHP-treated cells. At 48h, the percentage of apoptotic U-87 MG cells after exposed MEHP concentration like SK-N-SH was 60.67% compared with DEHP-treated cells was 8.18%. MEHP increased PARP cleavage and levels of cleaved caspase-3 in both cell types. MEHP also suppressed BCl-2 expression. The induction of autophagy is evidenced by the increased autophagosome formation and LC3-II expression in both cell types. MEHP induced ER stress-associated mRNAs, including PERK, ATF-4, CHOP, ATF6, IRE1α, spliced XBP-1 and CHOP. The increase of CHOP and spliced XBP-1 protein expression are found in MEHP-treated cells.
Discussion. The increase of DNA damage and proapototic signals lead to cell death after exposed to MEHP. The upregulation of markers of ER stress pathway by MEHP could lead to autophagy. The present study showed that MEHP is more toxic than DEHP on human neurons and astrocytes.

P562. Application of Bayse Theorem to augment machine learning predictions of Ames mutagenicity

Daniella James-News and Slade Matthews. Sydney Pharmacy School, The University of Sydney, Sydney, NSW, Australia



Introduction. Uncertainty accompanies all model predictions. Here we seek to augment the utility of toxicological predictive models through application of Bayes theorem and with information gleaned from collaboration with NIHS in Japan.
Aims. We aim to develop a machine learning model of Ames mutagenicity using published assay data and to use an unrelated dataset to develop informative Bayesian priors to further inform predictions made by the model.
Methods. Using a published dataset (Xu et al. 2012) we converted available SMILES molecular representations into Morgan fingerprints (radius 2) and modelled the relationship between molecular characteristics and mutagenicity reported in the Ames assay. Through analysis of the performance of this model we derived the Bayes factor for subsequent use in prediction augmentation. We grouped Xu data using Butina clustering and then applied the same clustering algorithm to the Honma dataset (NIHS) to calculate informative Bayesian priors. A holdout dataset from the Xu dataset was used to test the model (Figure). Following clustering predictions were made on the holdout dataset and the Bayes factor was applied.
Results. Predictions on the holdout dataset were able to be grouped into levels of certainty according to the informative Bayesian prior from cluster membership. The holdout dataset was within the chemical domain of the Honma dataset. Honma and Xu Datasets were assessed for overlap using their InChI keys and duplicates were removed. Predictions in the highest level of predictive confidence generally contained the lowest number of errors.
Discussion. Our results support the idea that a Bayesian approach to generation of predictive confidence is a plausible approach to delivery of predictive toxicological models for use in chemical regulation.

Xu, C., et al., In silico prediction of chemical Ames mutagenicity. J Chem Inf Model, 2012. 52(11): p. 2840-7.

P563. Fluoroquinolone ameliorates cadmium-induced pro-inflammatory cytokine production in human astrocytes

Suttinee Phuagkhaopong[1] and Pornpun Vivithanaporn[2]. [1]Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. [2]Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakan, Thailand.

Introduction. Cadmium poisoning can cause severe life-threatening impacts. Exposure of astrocytes to cadmium at low concentration 1 µM, which is about 50 times below safe level of cadmium intake recommended by FDA (45 µM), strongly showed an elevated expression and release of pro-inflammatory mediators, including IL-6 and IL-8 by activating through ERK and p38 MAPKs and NF-kB pathways (Phuagkhaopong et al, 2017). To date, there is no specific and effective approach to alleviate a progressive destruction of brain cells/tissue induced by cadmium toxicity. Fluoroquinolone (FQ), which are routinely used to treat lower respiratory tract infections and urinary tract infection, have been shown to exert additional immunomodulatory effects.
Aims. We hypothesized that FQ could suppress cadmium-induced neuroinflammation in human astrocytes.
Methods and Results. Co-treatment of cadmium at 1 µM and either moxifloxacin or levofloxacin at the concentration of 100 and 500 µM for 24 hours resulted in the inhibition of IL-6 and IL-8 secretion at different levels. Particularly, moxifloxacin has remarkable immunomodulatory effects on human U87 MG astrocytes. Furthermore, these FQs diminished intracellular cadmium uptake and decreased the resulting cadmium-induced cell death in human astrocytoma U-87 MG cell lines.
Conclusion. Together, these findings support previous findings on anti-inflammatory effects of FQ and provide an evidence that FQ could alleviate the toxic of cadmium by either act as a chelating agents or directly possessing anti-inflammatory effects. In the future, FQ could be a potential therapeutic agent for ameliorating cell/tissue inflammation induced by cadmium and other heavy metals such as nickel.

Phuagkhaopong S et al (2017) Neurotoxicology 60:82-91.

#ASCEPT2023
www.asceptasm.com

P564. Acute toxicity of Thai PM 2.5 in zebrafish embryos

Pinnakarn Techapichetvanich[1], Apisada Jiso[2], Paisan Jittorntam[3], Phisit Khemawoot[2], Wittaya Pimtong[4], Pornpun Vivithanaporn[2]. Program in Translational Medicine[1], Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; Chakri Naruebodindra Medical Institute[2], Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakan, Thailand; Research center[3], Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; Nano Environmental and Health Safety Research Team[4], National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand.

Introduction. PM2.5 has been shown to induce more severe health effects than larger particulates. The average dust concentration in Thailand exceeded the standard that could be hazardous to human health. However, the effects of PM2.5 collected in Thailand on living organisms have not been studied. Zebrafish (*Danio rerio*) has been used as a model for studying human disease pathogenesis from environmental risks.
Aims. To investigate the acute toxicity and examine the underlying mechanisms of standard PM2.5 and PM2.5 collected at Chakri Naruebodindra Medical Institute in zebrafish embryos (Thai PM2.5).
Methods. Acute toxicity study of PM2.5 in zebrafish embryos is followed OECD test guideline no. 236. Zebrafish embryos were exposed to the chemicals at doses of 200-1,000 µg/mL for 96 hpf. Gene expression involved in oxidative stress, inflammation, and apoptosis were investigated.
Results. Standard PM2.5 and Thai PM2.5 dust induced mortality, malformations, and gene expression (oxidative stress, inflammation, and apoptosis) changed of zebrafish embryos. The LC_{50} values of standard PM2.5 and Thai PM2.5 were more than 1,483.27 µg/mL and 512.01 µg/mL, respectively. Thus, Thai PM2.5 caused more toxicity than standard PM2.5.
Discussion. The difference in toxicity from both samples in embryos is likely caused by the difference of dust components rather than polycyclic aromatic hydrocarbon and heavy metals. PM2.5 toxicity studies in zebrafish embryos can provide valuable information that may be useful to understanding the health hazards associated with PM2.5 exposure in humans, particularly in terms of its effects on early development and potential long-term health consequences. This information can be applied to human health risk assessment and environmental policy decisions.

P600. The Role of Interleukin-18 Receptor Accessory Protein in Hypertensive Chronic Kidney Disease

Buddhila Wickramasinghe[1], Vivian Tran[1], Jake Robertson[1], Tayla Hughes[1], Maeve O'Keeffe[1], Henry Diep[1], Maria Jelinic[1], Grant Drummond[1] and Antony Vinh[1]. Department of Microbiology, Anatomy, Physiology and Pharmacology, Centre for Cardiovascular Biology and Disease Research[1], La Trobe University, Bundoora, VIC, Australia.

Introduction. The pro-inflammatory cytokine, interleukin-18 (IL-18), is elevated in patients with hypertension and chronic kidney disease (CKD). We have reported that genetic ablation and pharmacological inhibition of IL-18 prevents the development of experimental hypertension and renal inflammation. IL-18 binds to its cognate IL-18 receptor, which requires recruitment of the IL-18 receptor accessory protein (IL-18RAP) for activation.
Aim. To determine the effect of genetic deficiency of IL-18RAP (*Il18rap*$^{-/-}$) on deoxycorticosterone acetate and salt (DOCA/salt)-induced hypertension, renal hypertrophy and dysfunction.
Methods. *Il18rap*$^{-/-}$ mice were generated by Crispr-Cas9 on a C57BL/6 background. Male and female wild type (WT) and *Il18rap*$^{-/-}$ mice were anaesthetised under isoflurane, uninephrectomised (1K) and randomly assigned to be treated with either DOCA (2.4 mg/d, *s.c.* pellet) and high salt (0.9% in drinking water), or placebo and normal drinking water for 21 days. Systolic blood pressure (SBP) was measured weekly (tail-cuff), while transdermal glomerular filtration rate (tGFR) was assessed at baseline and endpoint. Mice were killed by CO_2 inhalation at day 21 and kidneys were harvested to assess renal hypertrophy (kidney weight: tibia length) and inflammation (flow cytometry).
Results. Baseline SBP was similar across sexes and genotypes (WT: 131±2 mmHg; *Il18rap*$^{-/-}$:133±2 mmHg). In WT mice, DOCA/salt caused an increase in SBP which peaked by day 7 and was sustained throughout the 21-day treatment period (161±3 mmHg). DOCA/salt also induced renal hypertrophy in WT mice (23 ± 1mg/mm), but surprisingly had no effect on either tGFR or the number of $CD45^+$ leukocytes in the kidney. For all parameters, responses were similar in males and females. The hypertensive response to DOCA/salt in male and female *Il18rap*$^{-/-}$ mice was indistinguishable from that in WT mice (169±4 mmHg). Yet despite this, *Il18rap*$^{-/-}$ mice appeared to be protected from DOCA/salt-induced renal hypertrophy (19 ± 1mg/mm).
Conclusion. Although *Il18rap*-deficiency was not protective against DOCA/salt-induced hypertension it did appear to limit renal hypertrophy. Further studies are required to determine the full extent of the renal protective effects of inhibiting IL-18RAP and thus its potential as a target for future therapies.

P601. Is there now evidence for rethinking statin dose?

Simon B Dimmitt[1,2], Michael C Kennedy[3], Genevieve M Gabb[4], Hans G Stampfer,[5] Jennifer H Martin[2,6]. Universities of Western Australia[1], Newcastle[2] & NSW[3], Flinders University[4], and Joondalup[5] and John Hunter[6] Hospitals, Australia.

Introduction. Epidemiologically, the incidence of coronary events continues to fall with lower total serum cholesterol, but below 6 mmol/L (low density lipoprotein, LDL-cholesterol below 4.5), total mortality levels out and then increases, in a 'J curve' manner.[1] HMG CoA reductase inhibitor (statin) doses have ranged 160-fold in efficacy and 25-fold in clinical outcomes trials. Efficacy plateaus with increased dose, whilst the incidence of multiple adverse events (AEs) continues to increase[1,2]. Minimum published effective statin dose (MED), pravastatin 5 mg (1/8 its ED50, the mean population oral dose that achieves a reduction in LDL-cholesterol by 50% of maximum possible, Emax) reduced both serum LDL-cholesterol and coronary events by about 15%. In the largest randomised placebo-controlled statin trials, the peak reduction in coronary events seen was 54% (around ED83), and in total mortality by 30% (around ED50)[2].
Methods. Clinical trials which recruited more than 4,000 coronary patients randomised to conventional (around ED50) or high (up to ED95) statin doses were sought through web search and PubMed.
Results. Mean baseline total cholesterol in the six studies was 4.6 mmol/L. Despite small reductions in coronary events, mortality was similar in patients randomised to conventional (e.g. atorvastatin 5- 10mg) vs higher doses.
Discussion. Increased AEs e.g. diabetes, renal or hepatic dysfunction, coronary calcification, as well as plateauing efficacy, may explain the failure to reduce coronary or total mortality with higher statin doses. Statin doses much above ED50 may be unnecessary, particularly when combined with management of other atheroma risk factors. Conventional doses have the advantage of greater tolerability (and thereby compliance) and safety.

1 Yi S-W, et al Sci Rep (2019) Sci Rep 9:1956-64
2 Dimmitt SB, et al (2018) Br J Clin Pharm 84:1128-12

P602. Comparing the anti-fibrotic potential of exosome-based therapies in human myofibroblasts in vitro

Jennie S Charoenphannathon[1], Chao Wang[1], Chrishan S Samuel[1]. Cardiovascular Disease Program, Monash Biomedicine Discovery Institute and Department of Pharmacology, Monash University[1], Clayton, VIC, Australia

Introduction. Fibrosis results from a failed wound-healing response to tissue injury and is characterised by the aberrant scarring of affected tissues. Currently-used treatments for fibrotic diseases mainly provide symptomatic management of disease progression but ineffectively reverse fibrosis, necessitating the development of alternative therapies. To this extent, mesenchymal stem cell (MSC)-derived exosomes (EXO) have emerged as a potential therapy for fibrotic diseases. MSC-EXO are extracellular vesicles secreted by MSCs that contain the immunomodulatory and tissue-reparative components of their parental cells, and are thought to provide the long-term benefits of MSCs well after MSCs are cleared from damaged organs post-transplantation. However, whether all sources of EXO induce anti-fibrotic effects and identifying the most effective and safe anti-fibrotic dose of each EXO still needs to be determined.
Aim. To determine the anti-fibrotic effects of EXO derived from various sources of MSCs in a human dermal myofibroblast culture model (as a stepping stone towards *in vivo* model testing).
Methods. BJ3 human dermal fibroblasts (HDFs) were seeded into 12-well plates ($1x10^5$ cells/well) and were either left untreated or stimulated with TGF-β1 (2ng/mL) to undergo myofibroblast differentiation (HDMFs) for 72h. Sub-groups of TGF-β1-stimulated HDMFs were also treated with human MSC-EXO from either adipose tissue (AD-MSC-EXO), bone marrow (BM-MSC-EXO) or umbilical cord blood plasma (UC-MSC-EXO), at a dose range of $1x10^7$ to $1x10^{10}$ particles (in duplicate wells/dose) for 72h (n=8/EXO tested). Equivalent aliquots of HDMF-derived protein were then assessed for fibrotic markers by Western blotting (α-SMA, collagen I, TIMP-1, TIMP-2) or gelatin zymography (MMP-2 and MMP-9).
Results. TGF-β1-induced a significant increase in α-SMA (HDMF differentiation) and collagen I expression, in the absence of having any marked effects on MMP or TIMP expression after 72h. These pro-fibrotic effects of TGF-β1 were only significantly attenuated by BM-MSC-EXO (but not by AD-MSC-EXO or UC-MSC-EXO), which prevented the TGF-β1-induced HDFM differentiation and collagen I deposition across all doses tested, and also promoted the MMP-2/TIMP-2 ratio at doses of $1x10^7$-$1x10^8$ particles, through a reduction in TIMP-2 expression levels after 72h.
Discussion. Only BM-MSC-EXO demonstrated significant anti-fibrotic potential in a HDMF culture model, particularly at a dose of $1x10^7$ particles, making this EXO a viable candidate for pre-clinical model testing *in vivo*.

P603. Interleukin-1 beta via Smad2 transcription factor promotes glycosaminoglycan chain synthesising gene expression.

Hirushi Kumarapperuma[1], Danielle Kamato[1,2]. School of pharmacy, The University of Queensland[1], Brisbane, QLD, Australia; Griffith Institute for Drug Discovery, School of Environment and Science, Griffith University[2], Nathan, QLD, Australia.

Introduction: Smad2 is a transcription factor typically downstream of the transforming growth factor beta (TGF-β) receptor 1 (TGFBR1). Smad2 has three regions: carboxy terminal and linker region that can be phosphorylated and an N terminal. Activation of TGFBR signaling leads to the direct phosphorylation of smad2 carboxy terminal whereas the Smad2 linker region undergoes regulatory phosphorylation mediated by serine/threonine kinases. Recent findings reveal that several receptor classes (e.g., G protein-coupled receptors and toll like receptors) through various intracellular mechanisms can activate Smad2 to elicit cellular responses. Therefore, we sought to elucidate the effect of inflammatory cytokine, interleukin 1 beta (IL-1β) on Smad2 activation in vascular cells.
Aim: Investigate the effect of inflammatory cytokine IL-1β on the activation of Smad2 transcription factor and downstream gene expression
Methods: The invitro model used vascular smooth muscle cells (VSMC). Protein expression of specific Smad2 residues were measured using western blotting and the mRNA expression of downstream gene expression was quantified using qRT-PCR.
Results: IL-1β treatment of VSMCs in a time and dose-dependent manner did not increase the phosphorylation of Smad2 in the carboxyl terminal. However, a rapid stimulation of phospho-Smad2 in the linker region was observed in as early as 15 minutes following treatment with IL-1β. Mechanistic studies with TGFBR1 and MYD88 inhibitors revealed that IL-1β mediated phosphor-Smad2 linker region occurred independent of TGFBR1 activation, and this response was completely inhibited by MYD88 inhibitor. A correlation of the signaling pathway was observed when assessing the mRNA expression of the glycosaminoglycan chain elongation gene (CHST11).
Discussion: We demonstrate that IL-1β via activation of only the linker region of the Smad2 transcription factor elicits proatherogenic responses. These findings open new avenues of research to investigate the role of IL-1β and Smad2 in other diseases.

P604. RAGE expression and function in myometrial arteries during gestational diabetes

Timothy V Murphy[1], Leo R Leader[1,2], Stephen D Horrowitz[1,2], Irit Markus[1], Lu Liu[1], Shaun L. Sandow[3], Anchita Kishan[1]. School of Biomedical Sciences, UNSW Sydney[1], NSW, Australia; School of Women's and Children's Health, Royal Hospital for Women[2], UNSW Sydney, NSW Australia; School of Health and Behavioural Sciences, University of the Sunshine Coast[3], Sippy Downs, Qld, Australia.

Introduction. Gestational diabetes (GD) is an increasingly prevalent complication of pregnancy. The hyperglycemia characteristic of GD results in the excessive plasma accumulation of advanced glycated end-products (AGEs) and tissue expression of their binding proteins, principally RAGE (receptor for AGEs) but also including AGER-1 and galectin-3.
Aims. This study aimed to explore the potential involvement of RAGE in maternal vascular dysfunction in gestational diabetes.
Methods. Small arteries (internal diameter ~200 µm) were dissected from pieces of myometrium and omentum obtained at term from normoglycemic (NG) women and others with GD. RAGE, AGER-1, NLRP3 and galectin-3 mRNA and protein expression in these vessels was investigated using rt-qPCR and immunofluorescence (IF), respectively. Functional studies examining the effects of AGEs on vasoreactivity of the arteries were performed using pressure myography.
Results. The mRNA expression of RAGE, AGER-1, NLRP3 and galectin-3 was not significantly changed in myometrial arteries from GD women (n = 8) compared with NG women (n = 9). IF studies suggested RAGE protein expression was increased in both smooth muscle and the endothelium of myometrial and omental arteries of GD women, while galectin-3 protein expression was also increased in the smooth muscle and endothelium of omental arteries only. Functional studies demonstrated that AGEs (0.1mg/ml) inhibited bradykinin-induced dilation of myometrial arteries from GD women (bradykinin pEC_{50} 6.57 ± 0.08) compared with NT women (7.30 ± 0.16; n = 4 for each, $P<0.05$). AGEs also induced contraction of the myometrial arteries in a time-dependent manner. Preliminary studies (n=1) suggest these effects of AGEs were prevented in the presence of the RAGE antagonist FPS-ZM1 (1 µM).
Discussion. These observations imply AGEs inhibit endothelium-dependent hyperpolarization of the myometrial arteries, as GD alone abolished NO/prostanoid-mediated dilation. AGEs also induced contraction of the myometrial arteries; combined with effects on vasodilation, AGEs may interact with RAGE to impair uterine blood flow in GD.

P605. Natriuretic peptide receptors in vascular smooth muscle cells in tissue versus culture

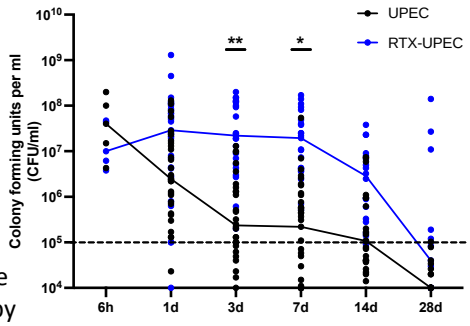
Christine Rager[1,2], Tobias Klöpper[1], Sabine Tasch[1], Michael Raymond Whittaker[2], Betty Exintaris[2], Andrea Mietens[1] & Ralf Middendorff[1]; Institute of Anatomy and Cell Biology, Justus-Liebig-University (JLU)[1], Giessen, Germany; Department of Pharmacy and Pharmaceutical Sciences, Monash University[2], Melbourne, VIC, Australia.

Introduction. Natriuretic peptide (NP) receptors NPR-1 and NPR-2 generate cyclic guanosine monophosphate (cGMP) upon binding of their respective ligands ANP and BNP, or CNP. cGMP mediates smooth muscle cell (SMC) relaxation.
Aim. To clarify the role of the NPs in vasculature, comparing primary cells and the corresponding intact tissue of origin (aorta tunica media), thus also assessing the potential effects of the isolation and culturing of vascular SMCs.
Methods. Aortic tissue of rats, housed at the Veterinary Faculty of the JLU was obtained. Procedures were conducted according to the guidelines of the German Animal Welfare Act and approved by the Committee for Laboratory Animals, JLU Nr. 577_M (01/2020 - 12/2022). With the help of a recently established reference gene* we quantified and compared the expression of cGMP/NP-related genes in tissue and cells by RT-qPCR. ELISA allowed the measurement of cGMP, released upon ANP or CNP treatment, hence an evaluation of the NPs at the functional level.
Results. The cGMP-specific ELISA confirmed the predominance of NPR-1 in intact vascular tissue ($p < 0.05$) at the functional level. In the cultured cells however, more cGMP was produced in response to CNP ($p < 0.05$) suggesting a predominance of NPR-2. The expression of NPR-1 remained at a similar level, but the expression of NPR-2 was elevated by ~5 in cultured cells, thus exceeding NPR-1 significantly. This matched the reduced cGMP production in response to ANP compared to CNP in cultured SMCs.
Discussion. Culturing alters cGMP/NP related gene expression and corresponding NP receptor activity in vascular SMCs. Possible explanations could be a general expression switch in all isolated SMCs or the predominance of a specific SMC subpopulation. Hence, total tissue or *in vivo* setups reflect the NP related signalling more realistically.

*Rager C, Klöpper T et al. (2023). Cells 2023, 12, 2135. https://doi.org/ 10.3390/cells12172135

P606. Neurogenic inflammation exacerbates urinary tract infection in mice.

Sharma H[1], Tay C[1], Bourlotos G[1], Stevens N[1,2], Sims S[2], Taylor S[1,2], Grundy L[1]
[1]Flinders Health & Medical Research Institute, Flinders University, Adelaide, SA; [2]South Australian Health & Medical Research Institute, Adelaide, SA.



Introduction: Neurogenic inflammation (NGI) arises due to pro-inflammatory peptide release by sensory nerves. NGI of the bladder causes urinary symptoms, including urgency and pain, and disrupts the urothelial barrier. Urothelial barrier disruption is a key risk factor for developing chronic urinary tract infections (UTIs). However, whether NGI directly impacts UTI susceptibility has yet to be determined. **Aims**: We aimed to use mouse models to study the contribution of NGI to UTI severity and persistence. **Methods:** Resiniferatoxin (RTX) was instilled into the bladder lumen of female C57Bl/6J mice over 3 consecutive days to induce neuropeptide release from afferent nerves and NGI. NGI was quantified by histological evaluation of bladder sections and phenotyping immune cells via flow-cytometry. 1-day post-RTX treatment, uropathogenic *E. coli* (UPEC $1x10^9$CFU/ml) was instilled into the bladder lumen via bladder catheterization to induce UTI. UTI persistence and bacterial load was determined by measuring colony forming units (CFU) from urine, kidneys, and spleen over 28 days. **Results**: RTX significantly increased T cell, B cell, NK cell, neutrophil and macrophage infiltration into the bladder wall and disrupted bladder wall integrity (n=5, $p<0.005$), indicative of NGI. Installation of UPEC induced UTI that gradually resolved over 28 days in untreated mice (Figure). In contrast, RTX treated mice exhibited significantly greater bacterial load in urine over time (Figure) (n=5-40, **$p<0.005$; *$p<0.05$) and increased CFU in the bladder wall and kidneys at 3-and 7-days post-infection (n=5-10, *$p<0.05$), indicating an exacerbated and prolonged UTI. **Discussion:** These results show that neurogenic inflammation can have a dramatic impact on the severity and persistence of UTI. These findings suggest that neuropeptides released from sensory nerves in the bladder may play a crucial role in the host-defence against UTIs. The mechanisms underlying these effects have yet to be determined but could be caused by interactions between neuropeptides and the immune cells that regulate infection clearance.

P607. Inhibition of the AT4R/ IRAP as a novel strategy to reduce renal fibrosis and injury in chronic kidney disease

S Chelvaretnam[1], PE Thompson[3], SY Chai[2], CS Samuel[1], RE Widdop[1], TA Gaspari[1], Biomedicine Discovery Institute and Dept of Pharmacol[1], Dept of Physiol[2]; Monash Institute of Pharmaceutical Sciences[3], Monash University, VIC, Australia.

Introduction. We have identified the angiotensin type 4 receptor (AT_4R), also known as the insulin regulated aminopeptidase (IRAP) as a promising new anti-fibrotic target, with pharmacological inhibition of IRAP reversing age-induced cardiac fibrosis. However, little is known about its role in the setting of renal injury and disease.
Aims. This study aimed to: 1) Investigate the effects of pharmacological inhibition or genetic deletion of IRAP in a high salt diet (HSD) induced model of kidney disease and 2) Compare the reno-protective effects observed with IRAP inhibition to current gold standard treatment with the angiotensin converting enzyme inhibitor (ACEi), Perindopril.
Methods. WT (C57Bl/6J) and IRAP KO mice (12 weeks old, n=6-10/group) were subjected to either normal drinking water (NDW) or 2% NaCl in drinking water (HSD) over a 12-week period. WT mice on a HSD were randomised to receive either: vehicle; IRAP inhibitor, HFI-419 (0.72mg/kg/day; s.c via osmotic mini-pump); or an ACEi, Perindopril (1mg/kg/day via drinking water) in the final 4 weeks of the experimental protocol.
Results. Mice fed a HSD presented with significantly increased IRAP expression (HSD+Veh=7.4±0.3% vs NDW=4.0±0.3%, $p<0.001$), interstitial fibrosis (HSD+Veh=4.1±0.3% vs NDW=2.7±0.3%, $p<0.001$) and glomerulosclerosis (HSD+Veh=2.3±0.2 vs NDW=1.7±0.2, $p<0.05$) which was accompanied by a trend in reduced kidney function. IRAP inhibition or gene deletion significantly reduced interstitial fibrosis (HSD+HFI-419=3.0±0.3%, HSD IRAP KO=3.0±0.2%; all $p<0.01$) and glomerulosclerosis (HSD+HFI-419=1.7±0.2, HSD IRAP KO=1.8±0.2, all $p<0.05$), accompanied by trends of improved renal function when it came to measurements of urinary urea as well as urinary and plasma creatinine levels. In contrast, Perindopril treatment had limited ability to improve either interstitial collagen expression (HSD+Perindopril=3.3±0.7%, $p>0.05$) or glomerulosclerosis (HSD+Perindopril=2.0±0.3, $p>0.05$).
Discussion. Targeting IRAP displayed an ability to regress fibrosis and glomerulosclerosis in a more long-term model of kidney disease, which was associated with trending improvements in renal function. Moreover, this study demonstrated that both pharmacological inhibition and genetic deletion of IRAP offered broader and greater reno-protection than treatment with the ACEi, perindopril.

P608. Characterising the impact of exercise and diet on the left ventricle in a mouse model of diet-induced obesity

Nimna Perera[1,2], Minh Deo[2], Oliver Fuller[1], Casey Egan[1], Miles De Blasio[2], Rebecca Ritchie[2], Mark Febbraio[1]
[1]Cellular and Molecular Metabolism Laboratory, [2]Heart Failure Pharmacology Laboratory, Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Parkville VIC, Australia

Background**:** Obesity is a significant contributor to cardiac mortality independent of its relationship with other cardiovascular risk factors. The detrimental consequences of obesity are due to both the associated cardiac structural and functional alterations. It is well-recognized that physical activity can be cardio-protective and improve metabolic homeostasis in obesity.
Aims: Investigate how the cardiac structure and function is altered in response to diet-induced obesity and long-term voluntary exercise.
Methods**:** At 6 weeks of age (wk), C57Bl6/J male mice commenced a chow or high fat diet (HFD). At 10 wks, mice were randomised to either a locked or unlocked running wheel. Following 20 wks of exercise training, a cardiac puncture was performed for blood collection and left ventricle (LV) collected for analysis. Magnetic resonance imaging was performed monthly to examine body composition and echocardiography at endpoint to assess LV function.
Results: Body weight was increased in the chow sedentary mice compared with HFD sedentary mice, but exercise had no effect on body weight. Fat mass was increased at the 3-, 4-, 5- and 6-month timepoint ($p<0.001$) following HFD feeding, and exercise attenuated this increase ($p<0.01$). Endpoint Doppler flow echocardiography indicated an increase in deceleration time ($p<0.001$), following HFD feeding. B-mode long axis indicated an increase in volume and area diastole ($p<0.05$) when comparing chow sedentary with chow exercise. Gene expression of hypertrophy markers; atrial natriuretic peptide and β-myosin heavy chain were decreased ($p<0.05$) when comparing chow sedentary with chow exercise mice and when coupled with B-mode analysis indicated left ventricular physiological hypertrophy. Non-fasted plasma triglyceride levels were not different following HFD feeding, but exercise reduced triglyceride levels ($p<0.05$). Furthermore, LV lipidomics indicated an increase in total triglycerides, free fatty acids, acylcarnitines, and a decrease in cardiolipins following HFD feeding, and exercise attenuated the decrease in cardiolipins.
Conclusion: Cardiac structure and function are influenced by diet and voluntary exercise training may attenuate changes following HFD feeding.

P610. Sex matters – impact of HFD and low-dose SGLT2i on rodent cardiometabolic phenotype

Abhipree Sharma[1], Minh Deo[1], Alex Parker[1], Nimna Perera[1], Anida Velagic[1], Dovile Anderson[2], David Shackleford[3], Miles De Blasio[1], Rebecca Ritchie[1]. Drug Discovery Biology[1], Monash Proteomics and Metabolomics Platform[2], Centre for Drug Candidate Optimisation[3],Monash University, Parkville, Australia

Introduction. Cardioprotection conferred by sodium glucose co-transporter 2 inhibitors (SGLT2i) differs based on sex and comorbidities (Sharma et al., 2023). The mechanisms behind these sex-specific effects remains unknown.
Aims. To investigate the effect of high fat diet (HFD) and the SGLT2i, dapagliflozin, on cardiometabolic phenotype in male and female mice.
Methods. 6-week-old male and female C57BL/6J mice commenced high fat diet (HFD; 60% kJ lipids) or chow. At 18 weeks of age, HFD mice were randomised to 8 weeks of dapagliflozin (target plasma concentration of 122.67 ng/mL) or vehicle (20% Trappsol) treatment via s.c. osmotic mini-pumps (n=8-12 per treatment group). Body weight, glucose and insulin tolerance, left ventricular (LV) systolic function and the plasma metabolome and lipidome were assessed.
Results. At study endpoint, the average plasma concentration of dapagliflozin in male and female HFD mice was 62.3±5.7 ng/mL and 92.8±20.1 ng/mL, respectively. Body weight was elevated in HFD females compared to chow (35.3±2.1 g vs. 28.9±0.9 g, $p<0.01$); dapagliflozin had no effect on body weight. HFD reduced glucose tolerance in females when compared to chow (area under the curve [AUC]: 999.3±114.1 vs. 653.1±58.3, $p<0.05$), while in males, dapagliflozin improved glucose clearance compared to vehicle (AUC: 857.0±66.3 vs. 1399.0±178.0, $p<0.05$). HFD-associated reductions in insulin tolerance in female mice were improved with dapagliflozin treatment (AUC: 614.2±26.5 vs. 777.5±20.5, $p<0.001$). LV systolic function was reduced in HFD males compared to chow (LV ejection fraction: 52.7±2.2% vs. 62.1±2.8%, $p<0.05$), with no effect of dapagliflozin on cardiac function. Although plasma lipid and metabolite profiles were altered with HFD across both sexes, dapagliflozin had minimal effect.
Discussion. The prediabetic phenotype associated with HFD and the metabolic effects of low-dose dapagliflozin differ in male and female mice, highlighting the need for further sex-specific interrogation of the cardiometabolic pathways affected by higher doses of SGLT2i in animal models with established diabetes.

Sharma A et al (2023) Lancet Reg Health West Pac 33:100692

P611. Lipoxin A_4 improves cardiac remodelling and function in diabetes-associated cardiac dysfunction.

Ting Fu[1], Muthukumar Mohan[2], Madhura Bose[2], Minh Deo[1], Eoin Brennan[3], Catherine Godson[3], Phillip Kantharidis[2], Rebecca H Ritchie[1*], Chengxue Qin[1*].[1]Drug Discovery Biology and [2]Department of Diabetes, Monash Univ, VIC; Australia; [3]Diabetes Complications Research Centre, Univ College Dublin, Ireland.

Introduction. Chronic inflammation is a key contributor to diabetic heart disease. A crucial aspect of a self-resolving inflammatory response is the production of locally active lipid mediators such as lipoxin A_4 (LXA_4) which initiate and promote the resolution phase[1]. However, the therapeutic potential of LXA_4 in diabetic hearts remains to be explored.
Aim. To investigate the therapeutic effects of adjuvant LXA_4 on diabetes-associated cardiac dysfunction.
Methods. 6-week-old male $ApoE^{-/-}$ mice were injected with vehicle/streptozotocin (55 mg/kg/day i.p. for 5 days) to induce diabetes. After 10 weeks of diabetes, mice were randomly allocated to receive either LXA_4 (5 µg/kg i.p.) or vehicle (0.02% ethanol) twice/week for a further 6 weeks. HbA_{1c} levels, left ventricular (LV) structure, and function were assessed.
Results. Diabetic mice exhibited elevated HbA_{1c} levels, and reduced body weight. Also, the higher macrophage content, elevated M1-like macrophage marker, increased collagen deposition, and upregulated expression of the lipoxin/formyl peptide receptors 2 (FPR2) axis, including (m*Fpr2* and m*Alox15*), were observed in their cardiac tissue. The diastolic dysfunction (e.g. prolonged deceleration time and the isovolumetric relaxation time) was evidenced in diabetic mice. Interestingly, administration of LXA_4 decreased the expression of M1 markers, m*Fpr2*, and m*Alox15*, reduced collagen deposition and improved diastolic function.

	Non-diabetic mice		Diabetic mice	
	Vehicle	LXA_4	Vehicle	LXA_4
Body weight (g)	32.3±0.4 (n=30)	31.3±0.4 (n=20)	25.6±0.7* (n=18)	24.3±0.7* (n=20)
HbA1c (%)	4.6±0.1 (n=19)	4.6±0.1 (n=13)	11.9±0.2* (n=18)	11.8±0.3* (n=20)
Cardiac collagen deposition (%)	1.6 ±0.2 (n=11)	1.4±0.3 (n=11)	3.1±0.4$^{\$\$}$ (n=9)	2.1±0.3$^{\#}$ (n=12)
Macrophage number (NO./0.43mm²)	11.7±1.1 (n=13)	12.4±1.2 (n=12)	18.2±2.6$^{\$\$}$ (n=9)	14.1±1.1 (n=9)
m*S100A9* (fold increase)	1.0±0.4 (n=17)	1.6±0.4 (n=9)	9.2±3.7$^{\$\$}$ (n=12)	2.2±0.6$^{\#\#}$ (n=12)
m*Fpr2* (fold increase)	1.0±0.2 (n=17)	0.8±0.1 (n=9)	4.0±0.9$^{\$\$\$\$}$ (n=12)	2.1±0.4$^{\#}$ (n=12)
A*lox15* (fold increase)	1.0±0.2 (n=17)	1.5±0.6 (n=9)	6.6±2.1$^{\$\$\$}$ (n=12)	3.2±0.8$^{\#}$ (n=12)
Deceleration time (ms)	20.4± 1.1 (n=12)	22.2±0.8 (n=16)	30.5±2.1$^{\$\$\$\$}$ (n=8)	25.6±0.7$^{\#\#\#}$ (n=14)
Isovolumetric relaxation time (ms)	22.9± 0.6 (n=12)	21.6±0.7 (n=16)	26.0±1.0$^{\$\$}$ (n=8)	21.5±0.8$^{\#\#\#}$ (n=14)

*P<0.0001 diabetic vs non-diabetic;$^{\$}$P<0.05, $^{\$\$}$P< 0.01,$^{\$\$\$}$P<0.001, $^{\$\$\$\$}$P<0.0001 vs non-diabetic + vehicle; $^{\#}$P<0.05, $^{\#\#}$P<0.01,$^{\#\#\#}$P<0.01 vs diabetic + vehicle, (2-way ANOVA, Fisher's post-hoc for multiple comparisons).

Discussion. We showed that LXA_4 protects against diabetic heart by reducing adverse remodelling and improving cardiac function. LXA_4-based therapy might be a novel approach to treating diabetic-associated heart disease.
[1]Hodges, R.R., Serhan, C.N., et al. (2017) Mucosal Immunology 10: 46–57.

ASCEPT Annual Scientific Meeting
Transformational Pharmacology & Toxicology
20–23 NOV 2023 International Convention Centre, Sydney
#ASCEPT2023
www.asceptasm.com

P612. Targeting the AT_4 receptor/IRAP reverses type 2 diabetes-induced cardiovascular dysfunction and remodelling.

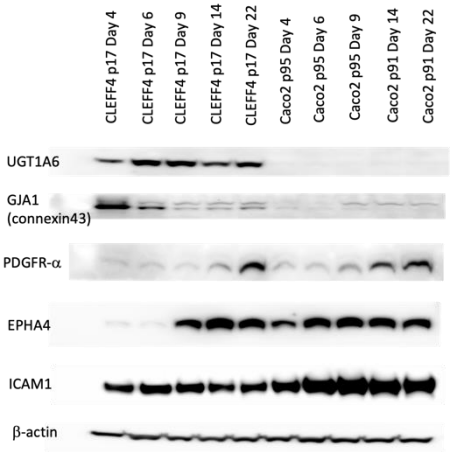
C Hollands[1], A Fitri[1], D Ferens[1], E Salimova[3], P Thompson[4], N Barlow[4], A Velagic[4], RH Ritchie[4], SY Chai[2], RE Widdop[1], TA Gaspari[1], Dept of Pharmacol[1], Physiol[2], MBI[3], MIPS[4], Biomedicine Discovery Institute, Monash University, Clayton, VIC.

Introduction. Cardiovascular diseases (CVDs) are the leading cause of death in patients with type 2 diabetes (T2D). Insulin regulated aminopeptidase (IRAP), also known as the angiotensin type 4 receptor (AT4R), has protective effects in a number of CVD models, however to date the effect in a type 2 diabetic model of CVD has not yet been explored.
Aims. Compare effect of chronic IRAP inhibition on cardio-renal pathology in a rat model of type 2 diabetes (T2D).
Methods. Male Sprague-Dawley rats (n=10/group, 8 weeks old) were placed on a high fat diet (HFD), 2 weeks later rats received 2 x daily injections of low-dose Streptozotocin (35mg/kg ip.) or citrate vehicle. Blood glucose and blood pressure (BP) were measured fortnightly. After 8 weeks of diabetes rats were administered either: vehicle, the novel IRAP inhibitor (IRAPi, 0.72mg/kg/day sc. via mini-pump, implanted under 5% isoflurane inhalation anaesthetic), the SGLT2 inhibitor Dapagliflozin (Dapa, 1mg/kg/day po.) or combination (IRAPi + Dapa) for a further 8 weeks. Cardiac function was assessed before and at end of treatment, following which rats were killed and tissues collected.
Results. No treatment intervention altered BP or glucose handling, although Dapa reduced overall blood glucose levels by ~50%. Diastolic dysfunction was established in all T2D groups after 8 weeks of diabetes (E/A ratio: T2D 1.09±0.04 vs Cit Veh: 1.38±0.10, n=10/group; p<0.05). Diastolic function was improved following 8 weeks of IRAPi, Dapa or combination treatment (E/A ratio: Cit Veh: 1.35±0.19; T2D: 1.02±0.15, p<0.05 vs Cit Veh; IRAPi: 1.14±0.12; Dapa: 1.20±0.21; Combo: 1.18±0.16. N=10/group). T2D significantly increased cardiac fibrosis (collagen % area 5.4±0.6, n=10) compared to citrate controls (2.9±0.2%, n=10; P<0.05). IRAPi and combination treatment reversed fibrosis more effectively than Dapa alone (collagen % area: IRAPi: 3.5±0.3%, p<0.05 vs T2D; Combo: 3.1±0.2%, p<0.05 vs T2D; Dapa: 4.1±0.4%. N=10/group). Similar protective effects were observed in the kidney and vasculature.
Discussion. IRAP inhibition exhibited cardiovascular and renal protective properties in a rat model of T2D. IRAP inhibition demonstrated better anti-fibrotic action with similar functional benefits when compared to the SGLT2 inhibitor Dapagliflozin. Combination therapy may have additional benefits, including reduction of hyperglycaemia. This study suggests that targeting IRAP provides an effective therapy against T2D-induced cardiovascular end-organ pathologies.

P613. Protein and RNA characterisation of rapidly maturing CLEFF4 and age matched parent Caco2 cells

Andrew Crowe[1,2]. Curtin Medical School, Curtin University[1], Perth, WA, Australia and Curtin Health Innovation Research Institute (CHIRI), Curtin University[2], Perth, WA, Australia.

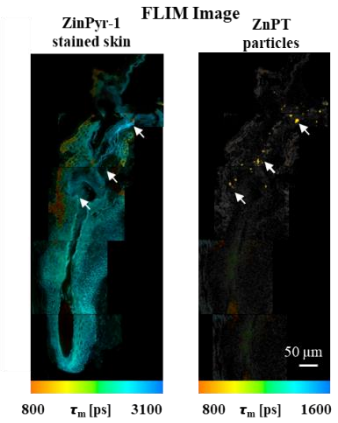
Introduction. The CLEFF4 sub clone from stock Caco2 cells has a unique property of being able to develop polarised cell monolayers with tight junctions & high P-glycoprotein (P-gp)/(ABCB1) expression 4x faster than the original cell line. Instead of being useful for transport studies 21-24 days after initiating culture, the CLEFF4 cell line matures in 5-6 days with tight junctions surpassing that of 3 week old Caco2 cells. This has enabled the CLEFF4 cell line to used for drug permeability assays, which predicts oral absorption for potential drug candidates, so important for pre-clinical drug development all within a single week. Aims. To define the unique properties of the CLEFF4 cell line compared to the original source Caco2. Methods. RNA samples were collected & analysed at days 4 & 7 of culture in triplicate over 3 years & had complete RNA transcriptome analysed by Genomics WA followed by quantitative analysis by the ranaseq.eu open bioinformatics platform. Protein was collected from day 4 to day 22 of culture. Results. Differential expression data from the FASTQ files have shown significant differences in expression in multiple genes involved with drug efflux, tight junctions, phase 2 metabolism and growth factors, which have been confirmed from protein determination that may hold the key to understanding accelerated human cell maturation. Conclusion. These gene expression results may be significant for other tissues beyond the gastrointestinal tract, and potentially for accelerated cell growth for the new field of laboratory grown tissues for organ replacement. The data also confirms the different genetic expression in CLEFF4 cells compared to Caco2 and the stable nature of the different expression over an extended period of multiple years.



GJA1 – Gap junction protein A1
ICAM1 –intracellular adhesion molecule 1
EPHA4 – EPH receptor A4
PDGFR-a – Platelet derived growth factor receptor alpha

P614. Critical evaluation of topical zinc pyrithione anti-fungal therapeutics

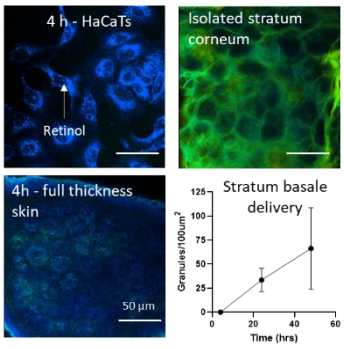
Sean E Mangion[1,2,3], Lorraine Mackenzie[1,2] Amy M Holmes[1,2], Michael S Roberts [1,2,4]
Therapeutics Research Centre[1], Basil Hetzel Institute, Adelaide, SA, Australia, UniSA: Clinical and Health Sciences[2], Adelaide, SA, Australia, Sydney Medical School,[3] Sydney, NSW, Australia City, Frazer Institute,[4] Brisbane, QLD, Australia



Introduction. Zinc pyrithione (ZnPT) is the most widely used anti-fungal in shampoo. It is incorporated as a suspension of fine particles, indicated for treating symptoms of dandruff and seborrheic dermatitis through inhibition of cutaneous Malassezia yeast. A key therapeutic challenge remains achieving adequate delivery of ZnPT to yeast colonisation sites, including the outermost skin layer (the stratum corneum) and the hair follicles. Aims. This investigation aimed to determine whether ZnPT could be delivered from commercial shampoo to the skin targets sites in dandruff at anti-fungal concentrations. Methods. A literature analysis was performed for a pooled assessment of ZnPT anti-fungal activity and topical delivery concentration. Complementing this, the optical properties of ZnPT were also characterised under single and two-photon excitation, enabling sensitive and specific assessment of the spatial delivery of ZnPT within the hair follicle by fluorescence lifetime imaging microscopy (FLIM). Results. The average (±SD) minimum inhibitory concentration (MIC) for ZnPT against two primary dandruff-related species, Malassezia globosa and Malessesia restricta, was 13.2 ± 11.7 ppm and 10.0 ± 7.0 ppm, respectively. ZnPT delivery to the stratum corneum was up to 500-fold higher than this target level, while delivery within the more protected yeast colonisation site of the hair follicle was lower and more variable, ranging from 20-fold lower to 35-fold higher than MIC levels. This was supported by FLIM imaging of follicular delivery, demonstrating either undetectable or limited ZnPT deposition at the entrance of the follicle (<100 µm) from three commercial shampoos, even after application of a 2-minute massage. Discussion. Together, this work shows that while the clinical efficacy of ZnPT shampoo is reflected in efficient delivery of ZnPT particles to the stratum corneum, there is further opportunity for optimising anti-dandruff shampoos by improving ZnPT follicular delivery.

P615. Visualising the topical delivery of retinoids

Sean E Mangion,[1,2,3] Amy M Holmes,[1,2] Lorraine Mackenzie[1,2] and Michael S Roberts[1,2,4]
Therapeutics Research Centre[1], Basil Hetzel Institute, Adelaide, SA, Australia, UniSA: Clinical and Health Sciences[2], Adelaide, SA, Australia, Sydney Medical School,[3] Sydney, NSW, Australia City, Frazer Institute,[4] Brisbane, QLD, Australia



Introduction. Retinoids are a diverse class of intracellular acting drugs that are first line agents in treating skin conditions such as acne and photo-damage. Understanding the sub-cellular spatial delivery of retinoids after topical application is critical for optimising safe and effective formulations. Aims. Here we aimed to determine if cellular retinoid delivery could be visualised using a time-resolved method, fluorescence lifetime imaging microscopy (FLIM). Methods. Fluorescence emission spectra and lifetimes were obtained for natural and synthetic retinoids under 2-photon excitation (720–920 nm). The HaCaT keratinocyte cell line was treated with retinoids (100 nM-20 µM) and live cell FLIM imaging performed up to 96 hrs (λex 740 nm, λem 420-460 nm). Six commercial formulations were optically characterised as per pure compounds, before application to fresh excised rat and human skin (10 mg/cm^2). FLIM images were acquired from the skin surface, granular, spinous and basal layers over 48 hrs. Results: Pure retinoids had 2-photon excitation maxima at 740 nm and could be distinguished by lifetime, ranging from 150 (tretinoin) to 2000 ps (retinol). Cell treatment resulted in distinct changes in lifetime, intensity and cell morphology (elongation and intracellular granulation) compared to growth control, especially with retinol (3.1-fold increase in intensity, 145 photons/pixel versus 450 photons/pixel, P<0.0005, and 2.6-fold increase in lifetime, 2500 ps versus 960 ps, P<0.0001). Commercial formulation components were visible using a FLIM-phasor approach and could also be distinguished from skin autofluorescence in excised tissues. Intracellular delivery was observed as high lifetime granules and was more extensive in rat than human skin. Discussion. FLIM is therefore useful for visualising retinoids in various systems from simple solution to keratinocytes *in situ* after topical application. This represents an important advance that will assist development of improved retinoid therapies for skin disease.

P616. Describing plasmapheresis (PLEX) at a single site: PLEX, the patient and impact on therapy.

Kiyan Hijjawi[1], Susan Welch[1], Jane Carland[2], Sophie Stocker[2], Graham Jones[3], Amanda McLaughlin[4], Karim Ibrahim[1], Fay Burrows[1], Lili Carlos[1], Debbie Marriott[5], Daniela Kocic[2], Richard Day[2], Vincent Seah[2], Peter MacDonald[6], Ian Sutton[7], Sam Rudham[8], Steven Morgan[8], John Moore[4]. Departments of 1.Pharmacy, 2.Clinical Pharmacology & Toxicology, 3.Pathology, 4.Haematology, 5.Microbiology, 6.Cardiopulmonary Transplantation, 7.Neurology, 8.Intensive Care, St. Vincent's Hospital, Sydney, NSW, Australia.

Introduction. Therapeutic plasma exchange (TPE) involves the removal of plasma (plasmapheresis) to treat various conditions. TPE can increase drug elimination which may affect treatment response. Limited data exists on the characteristics (including medications) of patients undergoing plasmapheresis.
Aim. To describe the characteristics of patients undergoing TPE, including medications prescribed.
Methods. A retrospective audit of patients undergoing TPE (2015-2020) at a metropolitan hospital was undertaken. Patient demographics, number of plasmapheresis sessions, indication for plasmapheresis and treating team were obtained from electronic records. Concomitant medications were obtained for a subset of patients (2019/2020). Data were summarised using descriptive statistics.
Results. Patients (n=204) underwent TPE; 54% were female with a median age of 52 years (range 19-81). There were 1414 TPE events, averaging 6.6 sessions per person. Common indications for TPE were antibody-mediated rejection (29%; 61/204), organ transplant desensitisation (32%; 66/204), blood type incompatibility (9%; 18/204), sickle cell disease (4%; 8/204) and myasthenia gravis (3%; 7/204). The average number of TPE sessions per person ranged from 1.1 for blood type incompatibility to 15.8 for sickle cell disease. Patients were most commonly admitted under lung transplant (39%; 79/204), haematology (23%; 47/204), heart transplant (13%; 28/204) and neurology (11%; 23/204). The subset of patients (n=52), for whom medication data were collected were 83% female with a median age of 55 years (range 24-81) and an average 6.6 sessions per person. Medications commonly taken were tacrolimus (52%; 27/52), mycophenolate (46%; 24/52), sulfamethoxazole/trimethoprim (50%; 26/52), methylprednisolone (34%; 18/52), heparin (27%; 14/52) and itraconazole (23%; 12/52).
Discussion. Plasmapheresis is indicated for a range of conditions. Many of the commonly prescribed medicines used by patients undergoing TPE require routine monitoring to ensure optimal drug exposure. Understanding how plasmapheresis influences medication disposition is required to facilitate precision dosing.

P617. Meropenem and ciprofloxacin combination regimens against isogenic *Pseudomonas aeruginosa* strains with different resistance mechanisms in a dynamic hollow fibre model

Alice Terrill[1], Kate E. Rogers[1], Carla López-Causapé[2], Wee L. Lee[1], Roger L. Nation[1], Antonio Oliver[2], Cornelia B. Landersdorfer[1]. Monash Institute of Pharmaceutical Sciences, Monash University[1], Parkville, VIC, Australia; Instituto de Investigacion Sanitaria Illes Balears[2], Palma De Mallorca, BALEARIC ISLANDS, Spain.

Introduction. *Pseudomonas aeruginosa* has a large armamentarium of mutational resistance mechanisms enabling resistance emergence during therapy against almost all antibiotics in monotherapy, including novel β-lactam/β-lactamase inhibitors.
Aims. To evaluate dosing regimens of meropenem (MER) and ciprofloxacin (CIP), alone and combined, against isogenic *P. aeruginosa* strains with different resistance mechanisms in a dynamic hollow fibre infection model (HFIM).
Methods. Four isogenic *P. aeruginosa* strains were: PAOD1 (spontaneous *oprD* mutation/loss of porin OprD), PAΔADmexR (*ampD* knock-out/AmpC overexpression and *mexR* knockout/MexAB-OprM overexpression), PAOD1ΔmexR and PAOD1ΔAD (other arrangement of combinations of the resistance mechanisms). Dosing regimens were: MER continuous infusion (CI, 6g daily dose against all strains, 12g daily dose additionally against MER-resistant strains), CIP intermittent infusions (400mg, 8-hourly [Q8] as 1-h infusions), and both combinations.
Results. All monotherapies resulted in regrowth with amplification of MER- and CIP-resistant subpopulations. The combination regimens suppressed total and resistant counts of PAOD1, PAΔADmexR and PAOD1ΔAD to below the limit of counting. Against PAOD1ΔmexR, the MER 6g CI + CIP regimen performed synergistically from 24h to 120h, while MER 12g CI + CIP was synergistic from 24h to 192h. MER-resistant counts displaying small colony morphology emerged from 72h and 168h with the respective combination regimens, plateauing at values similar to the control; CIP-resistant small colonies emerged from 216h with the low-dose combination only.
Discussion. Combination regimens of MER and CIP enhanced bacterial killing and suppressed regrowth and resistance of strains with one (PAOD1) or two (PAΔADmexR, PAOD1ΔAD) resistance mechanisms. Even against the double-resistant PAOD1ΔmexR substantial synergy occurred up to 120 or 192h. The performance of the combination regimens depended on the different resistance mechanisms present.

P619. Perceptions of third-year undergraduate pharmacology students on teamwork

Arani S Dasanayake[1], Yeong H Ling[1], Jennifer C Irvine[1], Betty Exintaris[2], Nilushi Karunaratne[2], Klaudia Budzyn[1]
Department of Pharmacology, Monash University, Clayton, VIC, Australia.[1] Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC.[2]

Introduction. Teamwork is increasingly valued across various fields during hiring processes. Teamwork supports both career success and positive workplace dynamics; however, there are particular challenges in fostering these skills in the tertiary education setting. One challenging aspect is ensuring investment and inclusion of all students, including international students. Language barriers, cultural differences, and different priorities and availabilities can hinder effective contributions to team activities (Sonnenschein, K., 2021). Thus, assessing student perceptions of teamwork is crucial to ensure that every student can benefit from teamwork.
Aims. To explore perceptions of teamwork in third-year undergraduate pharmacology students, with a focus on their identification of the main challenges faced by students.
Methods. Monash University students enrolled in the PHA3011 Principles of Drug Action unit (n=118) participated in a baseline teamwork assessment survey to measure their initial teamwork attributes and mindset. Subsequently, students from PHA3032 Neuro and Endocrine Pharmacology and PHA3042 Modern Drug Development units (n=73) anonymously shared their personal teamwork experiences through a survey. Additionally, three of these students joined a focus group for in-depth discussions on their perceptions of teamwork during their university studies.
Results. Findings from the study showed that 85% of the students strongly believe that teamwork is an essential job skill despite 35% of students still strongly preferring individual assignments. Thematic analysis of student responses highlighted that this preference may stem from various challenges to teamwork, including differing grade expectations, scheduling difficulties and lack of communication.
Discussion. Interestingly, the findings of this study highlight that students find generalized issues (perceived lack of effort of teammates, communication problems, divisive personalities), more of a concern when it comes to teamwork, rather than differences in cultural backgrounds and language barriers.

Sonnenschein. K (2021). *Journal of China Tourism Research*, vol:17(2), pp: 309-322.

P620. Nutrition counselling in pharmacy practice in Australia

Samar Elsayed[1], Jessica Pace[1], Joanna Harnett[1], Ingrid Gelissen[1]. School of Pharmacy, University of Sydney[1], Sydney, NSW, Australia.

Introduction. Chronic non-communicable diseases are forming an economic burden on individuals, health care systems and society. The World Health Organization recognizes diet as a major modifiable determinant of chronic disease and considers health promotion a top health service priority. Pharmacists are in a prominent position to implement health promotion strategies as they are the most approachable health care professionals with extended hours and no appointments needed. Counselling on lifestyle changes, including diet, in addition to providing medications is already taught in Pharmacy curricula, however it is unclear how effective this training is in practice.
Aims. To investigate what Australian registered pharmacists are currently providing to patients in terms of general nutrition counselling and whether pharmacists feel they have the skills and confidence to do so.
Methods. A 78-item quantitative survey was developed, based on our earlier work (Carter et al 2022), and delivered via RedCAP. Recruitment was predominantly via social media sites that were specific for registered Australian pharmacists. At the end of the survey, participants were invited to participate in a semi-structured interview.
Results. 107 complete surveys were collected between April and July '23 (12 male and 95 female). The survey showed that pharmacist already regularly counsel patients on general nutrition, with 16% reporting counselling daily, 32% counselling 2-3 times per week and 26% once per week. Most of the participants exhibited a positive attitude toward nutritional counseling, with 49% strongly agreeing and 31% somewhat agreeing that counselling patients about diet is an effective use of their professional time. In addition, 41% strongly agreed and an additional 40% somewhat agreed that counselling about diet should be part of routine care by all pharmacists. Most importantly, more than 80% of survey participants indicated that they felt that their pharmacy education did not include sufficient training in general nutrition. Twenty-one semi-structured interviews, probing more in-depth into the role of pharmacists in providing general nutrition counselling, were also completed and are currently being transcribed and analysed.
Discussion. The role of pharmacists in providing nutrition counselling will be discussed, including barriers identified in this study.

Carter C, Harnett J, Krass I and Gelissen I (2022) Curr Pharm Teach Learn 2022;14:1411-19.

P621. Using clinical case studies to build professional skills: A case study

Suong N T Ngo. The University of Adelaide, Roseworthy Campus, Adelaide, SA, Australia.

Introduction. While work relevant skills are typically acquired through placements in clinical practice setting, this study described a teaching module that utilised clinical based case studies to help students develop communication skills, teamwork and leadership skills and clinical decision making. In a recent study, critical thinking and practical knowledge and its application have been identified as a gap perceived by graduates in certain fields of education [1]. At present, limited data is available in the literature on the use of such module for building work relevant skills in undergraduate pharmacology teaching.
Methods. A total of 45 Veterinary Technology students who enrolled in Pharmacology, Pharmacy and Therapeutic II course in Semester 2 2022 at Adelaide Uni participated in the implemented practice-based learning module. Students worked in teams of 5-6 members, with a team leader appointed. Two specific tasks, self-learning and teamwork were required to be completed per a 3 hour- set time session. There was total 8 sessions per a 12 week- semester. Specific topics covered in these sessions included drug administration and dosage calculation, antimicrobial and antiparasitic drug therapy, cardiovascular drugs, variation in drug therapy/responses, gastrointestinal and neurological conditions. For self-learning, each student first solved an allocated clinical issue, presented as a case study. For teamwork, each team then met to discuss, negotiate and agree on how to solve the issue, and presented their decision and outcome to other teams by mean of a power point presentation. For each session, a different case study was allocated to each team. Evaluation and assessment of the implemented module were facilitated by peer assessment, in which each team presentation was judged and provided a score by other teams.
Results, Discussion. This teaching module required face-to-face attendance. However, the advantage is that the key recourses needed were the design/written of clinical based case studies, which are also useful generated resources for re-use later years, and MyUni online Course. Overall, students performed well in literature searching and problem solving, with their speaking skills and confidence improved greatly on completion of the module. Overall, the use of clinical case studies appeared to help students develop critical thinking skills and practical knowledge and its application. The implemented learning was found to increase student active engagement and participation.
[1] Aničić K.P., Munđar J.G., Šimić D. (2022). High. Educ., 1-21.

P622. Altered preference from online to face-to-face workshops does not influence academic outcomes

Elvan Djouma and Ross D. O'Shea. Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University, Bundoora, VIC, Australia

Introduction. Over the past 3 years (2021-2023), we have offered flexibility to students studying pharmacology subjects, where students could choose their mode of attendance for workshops (face-to-face [F2F] or online) using La Trobe's 'StudyFlex' model.
Aims. The aim of this study was to investigate changes in student preference for mode of study in a post-pandemic era and how this relates to academic performance.
Methods. We collected data from 559 students who enrolled in the subject 'General Principles of Pharmacology' in 2021, 2022 and 2023, and investigated preference for mode of study (whether students chose the F2F or online instance of the subject), workshop attendance, and subject results. The only difference between the instances was the mode of delivery of workshops (F2F or online). We compared academic performance for both F2F and online students, as measured by results on the individual assessments (two exams worth 60% of the total) and the overall subject mark, which included additional individual and team assessments. Workshop attendance for both groups was taken 15 minutes after the start of workshops, and the correlation between attendance and marks was analysed.
Results. Student preference for on-campus workshops increased over this period, from 37.7% in 2021 to 50.5% in 2023, while preference for online workshops decreased accordingly. Across this period, workshop attendance increased for the F2F cohort but decreased for the online cohort (Kruskal-Wallis ANOVA and Dunn's post-hoc test). Test marks and overall subject marks were highly correlated with workshop attendance, independent of attendance mode, but average marks were not different between the two cohorts in any year. Non-parametric Spearman correlations revealed a significant correlation between the percentage of workshops attended and both test marks and overall marks for students in both modes of attendance (all $P<0.05$).
Discussion. There has been a shift in preference for F2F workshops over online workshops from 2021 to 2023. Despite this, mode of workshop attendance had no significant effect on academic performance, but greater workshop attendance, independent of mode, was correlated with improved academic outcomes.

P623. Use of a baseline teamwork assessment tool in undergraduate pharmacology

Lynette B Fernandes[1], Nilushi Karunaratne[2], Betty Exintaris[2]. School of Biomedical Sciences, The University of Western Australia[1], Perth, WA, Australia; Faculty of Pharmacy and Pharmaceutical Sciences, Monash University[2], Melbourne, VIC, Australia.

Introduction. "Being a team player is the most valuable quality a person should develop in order to thrive in the world of work and life" (Lencioni, 2016). However, teamwork does not occur merely as a consequence of placing people together (Salas et al., 2008).
Aims. To explore underlying team member attributes of undergraduate pharmacology students.
Methods. In 2022 and 2023, 2nd year pharmacology students (265) were invited to complete the Monash University Teamwork Assessment Tool in Week 1 of semester. This Likert-scaled survey included items to: 1) explore growth mindset for the 'kind of person' someone is; 2) explore growth mindset for intelligence; and 3) determine self-awareness. Students received a personalised report of their results including mindset scales scores; how they rated themselves on humility, hunger for success, and people smarts; and suggestions for further development. (Monash University HREC Approval 36122)
Results. Over two years, 80% of students completed the Tool (n=211). Students largely agreed that the 'kind of person' someone is (55-77% Agreement) and one's intelligence can be changed (67-82% Agreement). Most students usually were glad to share credit for team accomplishments (85%), were aware of how their words and actions impact others on the team (74%) and offer and accept apologies graciously (71%). However, fewer students usually do more than what is required in their own job/role (38%) or demonstrate an interest in the lives of their teammates (43%). Overall, mindset scale and Ideal Team Player scores indicate students had a growth mindset with some fixed ideas about the 'kind of person' someone is and a strong growth mindset about intelligence. Most students have some work to do around humility, hunger for success and people smarts.
Discussion. The personalised report could empower students to develop their teamwork skills. Findings from this study will be used to develop resources to support the development of teamwork skills in undergraduate students.
Lencioni PM (2016) The Ideal Team Player. Hoboken, New Jersey, Jossey-Bass.
Salas E et al. (2008) Hum Factors 50:903-933

P624. Scaffolding resilience skill development across an undergraduate Pharmacy curriculum

Betty Exintaris, Nilushi Karunaratne, Annie Chen-Chen, Anisha Kaur, Abdullah Jaafar and Kirsten Galbraith.
Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia.

Introduction. The Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), Monash University Australia (MUA) and Malaysia (MUM), identified the need to introduce the topic of resilience to undergraduate students, and to give the students multiple scaffolded opportunities to develop their resilience such that they are equipped with the necessary skills when facing challenging situations in their student and personal lives, and as health care professionals.
Aims. To design, develop and implement a resilience curriculum across years 1-4 of the Bachelor of Pharmacy (Hons) / Masters of Pharmacy degree.
Methods. In 2021, an internal Faculty education grant was received to support the development of the resilience curriculum. Funds were used to appoint a practitioner educator (hospital pharmacist) to support the design and development of the curriculum. The expert team assembled a draft of evidence-based key features that the resilience curriculum should include but was not limited by the scope and expertise of the practitioner educator.
Results. The resilience curriculum was strategically scaffolded into the Skills Coaching program (at both MUA and MUM). The focus of each resilience session varied from 'What is resilience?' (Year 1) to 'Self-talk' (Year 2), 'What is flexible Thinking?' (Year 3) to authentic scenarios where medication errors have been made in Year 4 'Community pharmacy dispensing error case study' The students had the opportunity to reflect on each of the resilience sessions receive personalised feedback from their skill's coach. The resilience curriculum was piloted for the first time in 2022. Pharmacy students across both MUA and MUM were receptive to exploring resilience training further and felt that it is a useful skill that will aid them in becoming well-rounded health professionals when they enter the pharmacy workforce.
Discussion. A resilience curriculum was designed, developed and implemented into the Skill's coaching program as part of the Pharmacy curriculum which focuses on skill development. Feedback obtained from staff and students this year will be used to further develop the curriculum moving forward.

P625. Streamlining Nursing Home Discharges from the Acute Hospital Setting

Angelo A Bombuwelage Don[1], Nilushi Karunaratne[2] and Betty Exintaris[2] Slade Pharmacy[1], Richmond, VIC, Australia; Faculty of Pharmacy and Pharmaceutical Sciences, Monash University[2], Melbourne, VIC, Australia.

Introduction. Patients discharging to a Residential Aged Care Facility (RACF), post the acute Hospital Setting, require prompt and accurate medication supply to ensure continuation of safe healthcare. Ideally, the Clinical Ward Pharmacist (CWP) liaises with the RACF that the patient is to be transferred to and communicates with the affiliated local Community Pharmacy (CP) prior to discharge; unfortunately, it is current practice amongst the CWPs to manually pack the patient's medications into Webster Packs. These packs are mostly not used by the staff at the RACF once the patient arrives, stating that they instead rely on the supply of medication from their affiliated pharmacy, leading to the destruction of the pre-packed medications.
Methods. To develop a standardised document outlining the individual requirements of nursing homes on the day of discharge. Results. A structured table was created, containing most RACFs within the state of Victoria (n=312) and important information (affiliated community pharmacy and what was expected from the CWP on discharge). Procurement of this information involved calling local nursing homes, requesting the contact details of the pharmacy that primarily services them, and establishing the CP's preference from the hospital on day of discharge for their residents. In calling the pharmacies, other nursing homes that they service were also obtained hence expanding the list. Furthermore, nursing homes part of a larger organisation were all included to ensure completeness of the protocol. Following implementation, immediate benefits were noted wherein a 30-minute RACF discharge had been streamlined to a quick 5 minute read of the protocol and applying the appropriate process to the affiliated CP.
Discussion. A standardised document outlining the individual requirements of nursing homes on the day of discharge was created. By reducing the time, it takes to process nursing home discharges, CWPs may better manage their day with other pertinent tasks. This protocol will be trialled on a Long-Stay Surgical/General Medicine ward, before branching out to be used by social workers hospital wide, with the potential for inter-hospital use to ensure patient centred care is delivered effectively and efficiently.

P626. Accessing lecture recordings as a supplement or a substitute to lecture attendance.

Sheila A Doggrell, School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, QLD

Introduction. Educators often advise students to access lecture recordings as a supplement, rather than as a substitute, for attending lectures. The basis for this advice is not clear.
Aims. To determine supplementary or predominantly substitute access of lecture recordings in a pharmacology course, and the effect on the examination mark, and of gender. The examination is of the lecture material.
Methods. Lecture attendance and accessing lecture recordings were collected from lecture 2-11. Students who attended one or less lectures were grouped as predominantly substitute accessors of lecture recordings.
Results. Among the 92 consenting students, lecture attendance was low, 15%. Students who accessed lecture recordings predominantly as a substitute for lecture attendance, rather than a supplement, had significantly lower attendance (2% vs 72%), higher lecture recording access (61% vs 19%), and lower marks in the exam (78% vs 85%). Sixty percent of the students were female. Lecture attendance and examination marks were similar between genders. Lecture recording access was higher for females than males (56% vs 32%). Female students who accessed lecture recordings predominantly as a substitute for lecture attendance had significantly lower attendance (2% vs 64%), higher lecture recording access (63% vs 28%), and lower marks in the exam (76% vs 85%). Only a small number of male students accessed lecture recordings as a supplement to lecture attendance (n = 6, predominantly substitute n = 31). These six supplementary male students had higher attendance (87% vs 2%), lower lecture recording access (3% vs 37%) and higher examination marks (85% vs 78%) than the predominantly substitute accessors.
Discussion. As many students only attended the first one or two lectures, it is possible that after attending, they decided whether they were going to continue attending or use accessing lecture recordings as a substitute for attending. Some students may have made the wrong choice, as students who attended lectures and used the recordings supplementarily had better outcomes in the exam. This data provides a basis for advising students to attend lectures, and access lecture recordings supplementarily.

P627. Innovative educational program to enhance pharmacy student knowledge and confidence in pharmacogenomics

Ruby Soueid[1], Toni JF Michael[1], Rose Cairns[1], Kellie A Charles[1], Sophie L Stocker[1,2]. School of Pharmacy, Faculty of Medicine & Health, The Univ of Sydney[1], Sydney, NSW; Dept of Clin Pharmacol & Toxicol, St. Vincent's Hosp[2], Sydney, NSW.

Introduction. The lack of uptake of pharmacogenomics (PGx) in clinical practice is associated with poor knowledge and confidence. Pharmacists play a key role in the implementation of PGx services. Innovative pedagogical approaches are required to develop the competence of pharmacy students, to steward PGx-guided medication management.
Aims. To evaluate the impact of an innovative educational program on pharmacy student knowledge, attitudes and confidence in PGx.
Methods. Second-year pharmacy students (n = 278) at the University of Sydney participated in a PGx module that consisted of lectures (5 hours) and a workshop (2 hours) with case-based learning. Student knowledge, attitudes and confidence in PGx were assessed before and after delivery of the education using the same survey. The survey consisted of 41 questions that were scored using a 6-point Likert scale. Results from surveys were compared using descriptive statistics. Focus groups were also conducted to gain further insights into survey responses.
Results. Pre- and post- survey response rates were similar (96% and 90%, respectively). Although 80% of students reported improvements in their PGx knowledge and skills, 83% requested more activities in the application of PGx information, using case-based learning. 84% of students felt "slightly, somewhat or extremely comfortable" in interpreting PGx test results. However, only 72% reported being confident in designing a PGx-guided dosing regimen. Communication of PGx-guided recommendations to other healthcare professionals as part of interprofessional collaboration improved by 41% to 78% . Qualitative analysis highlighted student interest in using role play simulations and personal PGx testing to support their learning. However, 58% of students expressed concerns about the ethical use of their own PGx data. Objective measures in knowledge will be assessed in final examinations.
Discussion. This study demonstrates that innovative pedagogy, specifically interactive case-based learning, can enhance PGx knowledge and confidence in applying new PGx knowledge in pharmacy students. Problem-based activities and self-PGx testing may further support student learning. Further exploration of the ethical implications associated with personal PGx testing is required.

P628. Novel treatments targeting neurodegeneration and inflammation attenuate repetitive mild traumatic brain injury induced injury in a preclinical model

Matthew Hiskens[1,2] , Katy Li[1], Anthony G. Schneiders[1], Rebecca Vella[1], Andrew Fenning[1]. School of Health, Medical and Applied Sciences, CQUniversity[1], North Rockhampton, QLD, Australia; Mackay Institute of Research and Innovation, Mackay Hospital and Health Service[2], Mackay, QLD, Australia.

Introduction. Concussion trauma or repetitive mild traumatic brain injury (rmTBI) is a major health issue that causes increased inflammation and oxidative stress in the brain. It is now linked to longer term, more severe neurological disorders, for which there is currently no effective treatment. Neurodegeneration and microstructure and cellular changes follow the inflammatory and oxidative burst which also triggers a cascade of neuroexcitatory signalling causing further neuronal damage. Our research has developed a preclinical mouse model which reliably induces rmTBI and CTE. Novel treatments such as minocycline (M) and acetyl-L-carnitine (ALC) have been postulated to provide neuroprotection via anti-inflammatory mechanisms and by inhibiting excitotoxicity.
Aims. To prevent rmTBI induced damage in a preclinical model using M and ACL targeting inflammatory, oxidative stress and excitotoxic pathways.
Methods. Adult male C57BL/6J mice were allocated to sham, rmTBI, M + rmTBI or ALC + rmTBI groups. 15 rmTBIs were administered across 23 days using a modified weight drop model. Neurological testing and spatial learning and memory assessments via the Morris Water Maze (MWM) were undertaken at 48 hours and three months. RT-PCR analysis of the cortex and hippocampus was undertaken for MAPT, GFAP, AIF1, GRIA, CCL11, TDP43, and TNF genes.
Results. Both M and ACL showed functional neuroprotection (spacial, righting reflex and MWM) compared to the untreated rmTBI animals. These functional changes were also associated with prevention of gene upregulation post rmTBI in the cortex and hippocampus with increases in GRIA1, MAPT, TNF and GFAP all partially prevented by M or ALC treatment.
Discussion. Novel treatments like M and ALC show promise and provide further mechanistic understanding of the cellular damage which occurs to the brain following rmTBI. These neurodegenerative cascades are linked to functional impairment and potentially provide an avenue for further drug development and a deeper understanding of the pathophysiology of this condition.

P629. Osteoarthritis, opioids, & polypharmacy: deprescribing outcomes in middle-aged male & female mice

Charlie Gregson[1], John Mach[1], Carina Blaker[1], Bryony Winters[1], Yo Otsu[1], Neda Assareh[1], Kevin Winardi[1] Sarah Hilmer[1]. Kolling Institute, Northern Sydney Local Health District and Faculty of Medicine and Health, University of Sydney, St Leonards, NSW, Australia.[1]

Introduction. Polypharmacy (using ≥5 medications) is more common in multi-morbid individuals, and is associated with functional and cognitive deficits. Most patients with osteoarthritis have comorbidities, and many take long-term opioids to manage pain, despite safety issues and little evidence of long-term efficacy. Deprescribing is the supervised withdrawal of unnecessary or harmful medications to improve patient outcomes. Aims. To assess the effect of oxycodone, polypharmacy and deprescribing oxycodone in middle-aged, osteoarthritic mice. Methods. At age 12 months, male and female C57BL mice (n=12-15 per group) had post-traumatic osteoarthritis induced and were administered either control, oxycodone alone or in a polypharmacy regimen (oxycodone, citalopram, simvastatin, oxybutynin, and metoprolol). After 6 weeks half of these mice were tapered off (deprescribed) oxycodone. There were two control (no treatment) groups, one with and one without osteoarthritis. The open field, light-dark box, nest scoring, and acetone evaporation test were used to measure anxiety (midzone distance percentage) and locomotion (distance travelled), anxiety (time in light), functional activity and the presence of cold allodynia (pain), respectively. Testing was performed at baseline and after injury at day 3, week 6 and week 12. Results. Preliminary data found polypharmacy increased anxiety in the open field for females ($p<0.05$) and in the light-dark box for males ($p<0.05$). There were no significant differences between any groups in distance travelled. Male and female polypharmacy groups had poorer nesting scores than control ($p<0.0001$) without significant change after deprescribing oxycodone. Male control osteoarthritis had poorer nesting score than no-osteoarthritis ($p<0.01$). On the acetone evaporation test, males displayed more pain behaviour on oxycodone compared to corresponding controls with osteoarthritis ($p<0.05$), and the deprescribed group showed less pain behaviour than mice continuing oxycodone ($p<0.001$). In females there was no significant difference in the acetone test with osteoarthritis, oxycodone, polypharmacy or deprescribing oxycodone. Discussion. Long-term oxycodone may induce cold allodynia in males, which is reduced on deprescribing. In both males and females, polypharmacy decreases function and increases anxiety, which is not significantly reversed 6 weeks after deprescribing oxycodone.

P630. Modulation of neuronal ion channels by metabolites of cannabidiol

Jess Wigmore[1,2], Maisie Johnson [1,2], Marina Junqueira Santiago[1], Mark Connor[1]. Macquarie Medical School, Macquarie University, Sydney, NSW, Australia; University of Exeter Medical School, University of Exeter, Exeter, England

Introduction. Cannabidiol (CBD) is a component of medicines for multiple sclerosis and epilepsy, and it is also consumed by a significant proportion of the population in Cannabis preparations. CBD is extensively metabolized, and the effects of CBD metabolites on potential pharmacological targets of CBD is largely unknown.
Aims. This study aimed to investigate the effects of some primary CBD metabolites on ion channels reported to be modulated by CBD.
Methods. In this study we examined the effects of 7-OH CBD, 7-COOH CBD and 6α-OH CBD on TRPV1, TRPA1 and $Ca_V3.1/2$ (T-type) channels using a fluorescent assay of intracellular Ca ($[Ca]_i$) to measure drug modulation of human channels stably expressed in HEK293FlpInTREx cells. Statistical comparisons between drugs were made by One-way ANOVA followed by Dunnett's multiple comparison test.
Results. CBD and metabolites activated TRPA1 at concentrations greater than 10 µM, CBD and 6α-OH CBD (30 µM) produced elevations of $[Ca]_i$ not different to the maximally effective concentration of cinnamaldehyde (CA, 300 µM), while 30 µM 7-OH CBD or 7-COOH CBD produced elevations of $[Ca]_i$ that were significantly less then CA ($P < 0.05$, n=6). The TRPA1 antagonist HC-030031 (30 µM) inhibited these elevations of $[Ca]_i$. CBD and metabolites did not activate TRPV1, however, 7-OH CBD (10 µM, 30 µM) and 6α-OH CBD (30 µM), but not CBD, inhibited the response to 10 nM capsaicin ($P< 0.05$, n=6). At 10 µM, 7-OH CBD and 6-α-OH CBD were more effective ($P < 0.05$) than CBD at inhibiting the elevation of Ca produced by activation of $Ca_V3.1$ (88 ± 8%, 80 ± 4%, 25 ± 25% inhibition, respectively) and $Ca_V3.2$ (79 ± 4%, 52 ± 13 %, 33 ± 14% inhibition respectively).
Discussion. The CBD metabolites 7-OH CBD and 6α-OH CBD were more effective than CBD at inhibiting T-type Ca channels and TRPV1 activation, and all were TRPA1 agonists. It seems unlikely that actions at TRP channels contribute to the pharmacotherapeutic profile of CBD in people, but modulation of voltage-gated ion channels might, and the more polar CBD metabolites are more effective than CBD in modulating T-type channels in a naturalistic assay of Ca channel function.

ASCEPT Annual Scientific Meeting
Transformational Pharmacology & Toxicology
20–23 NOV 2023 International Convention Centre, Sydney
#ASCEPT2023
www.asceptasm.com

P631. Structural Insights into Positive Allosteric Modulation at the M_4 mAChR

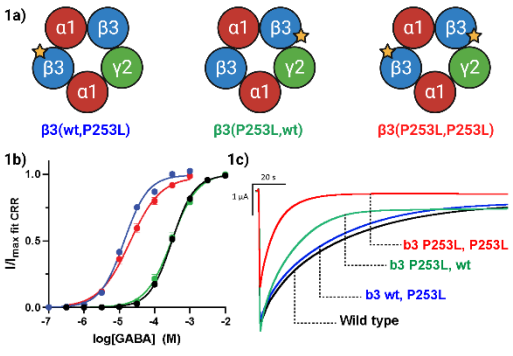
Michaela Georgia Kaoullas[1, 2], Celine Valant[1], David Thal[1, 2], Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences[1], Melbourne, VIC, Australia; ARC Centre for Cryo-Electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences[2], Melbourne, VIC, Australia

Introduction. The M_4 muscarinic acetylcholine receptor (mAChR) has emerged as an exciting new target for the treatment of CNS disorders, such as schizophrenia. A major challenge is the selective activation of the M_4 mAChR due to the mAChRs having highly conserved orthosteric binding sites. We have recently determined cryogenic-electron microscopy (cryo-EM) structures of the M_4 mAChR bound to a new generation of selective positive allosteric modulators (PAMs), compound XY6. The PAM XY6 has a 10-fold improved binding affinity versus the previous generation of M_4 PAMs such as LY2033298 and VU0467154. Understanding why XY6 has improved binding properties in comparison to previous generations of M_4 PAMs will facilitate the design of future M_4 mAChRs based therapeutics.
Aims. Determine how residues in the allosteric site of the M_4 mAChR contribute to the binding affinity (pK_B), binding cooperativity (α), allosteric agonism (τ_B), and functional cooperativity ($\alpha\beta$) of XY6 with the agonist ACh.
Methods. Using site-directed mutagenesis, we replaced key residues in the allosteric region of the M4 mAChR with alanine and generated stably expressing cells lines (Flp-In CHO cells). The pharmacological properties of XY6 and ACh were determined in radioligand binding assays and BRET-based G protein activation assays (TruPATH).
Results. We found that mutations of residues Y89, F186, and W435 into alanine significantly reduced the binding affinity of XY6 (pKB) and the transmission of cooperativity towards ACh (α_{ACh}) compared to the WT M_4 mAChR. Additionally, we found that the degree of ago-PAM activity was significantly reduced.
Discussion. These findings suggest that all three aromatic residues, Y89, F186, and W435 are key contributors to XY6's allosteric binding mechanism. Critically, this also suggested that XY6 is less sensitive to mutation of these aromatic residues' compared to previous M_4 PAM scaffolds. These data can inform future structure-activity relationship studies to enhance the development of therapeutically beneficial M_4 mAChR PAMs.

P632. Epilepsy-causing proline paralog variant in $GABA_A$ receptor display distinct functional properties between subunits.

Sze Hon Kan[1], Nazanin A. Mohammadi[2], Vivian W. Y. Liao[1], Susan X. N. Lin[1], Nathan L. Absalom[3], Rikke S. Møller[2], Mary Chebib[1] and Philip K. Ahring[1]. Brain and Mind Centre, The University of Sydney[1], Camperdown, NSW, Australia; Department of Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Centre[2], Dianalund, Denmark; Discipline of Medical Sciences, Western Sydney University[3], Campbelltown, NSW, Australia.

Introduction. Paralogous variants in the *GABRA1* (p.P260L), *GABRB2* (p.P252L) and *GABRB3* (p.P253L) genes have been found in patients with severe epilepsy. To understand how this proline variant contributes to pathogenicity of the disease, its functional effects were evaluated.
Aim. To identify functional differences in the GABA dose-response and desensitisation of $GABA_A$ receptor variants.
Methods. Concatenated $GABA_A$Rs containing either single or double $\alpha1^{P260L}$, $\beta2^{P252L}$ or $\beta3^{P253L}$ (Figure 1a) were constructed. GABA sensitivity (EC_{50}) was determined by performing dose-response curves (Figure 1b) and receptor desensitisation was determined by calculating the decay rate constant and steady state/baseline current ratio following prolonged GABA application (Figure 1c).

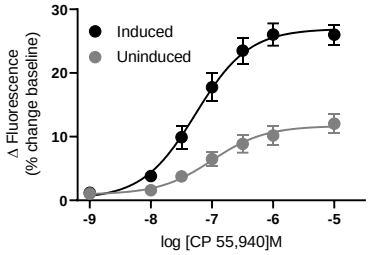


Results. All variants except for $\beta3^{P253L/wild\ type}$ had a significant increase in GABA sensitivity displaying 25-30 times increase in GABA EC_{50} compared to wild type (WT) (Mean variants vs WT EC_{50} range (µM): 5.5-110 vs. 200-330). In addition, homozygous (double variants) receptors displayed a loss of maximum current. While $\alpha1^{P260L}$ variants did not show significant receptor desensitisation, all six $GABA_A$Rs with β2 and β3 variants significantly desensitised upon prolonged GABA exposure (Mean variants vs WT decay rate constant range (s^{-1}): 0.033-0.089 vs. 0.02-0.023).
Discussion. This study shows that the proline paralog variant found in $GABA_A$ receptor subunits displays distinct functional characteristics. These characteristics depend on the specific subunit involved, and whether the variant is present on one (heterozygous) or both subunits (homozygous) of the same receptor. This functional assessment is valuable to guide clinicians' treatment approaches for patients living with severe epilepsy.

P633. A new system to determine Cannabinoid receptor-2 (CB2) ligand efficacy

Tahira Foyzun[1], Marina Junqueira Santiago[1], Michael Kassiou[2], Annukka Kallinen[2], Mark Connor[1]. Macquarie Medical school, Macquarie University[1], Sydney, NSW, Australia; School of chemistry, University of Sydney[2], Sydney, NSW, Australia.

Introduction. CB_2 is emerging as a promising therapeutic target for treating conditions including chronic and inflammatory pain, cancer, and neurological disorders. Understanding the efficacy of CB_2 ligand is crucial for further drug design and development.



Aims. To establish a robust and simple system using tetracycline-regulated expression (T-Rex) of CB_2 so that we can use the Black and Leff (Black and Leff, 1983) operational model to estimate the operational efficacy (τ) of a range of selective CB_2 ligands.

Methods. A stable AtT20FlpInTREx cell line expressing CB_2 was generated and a fluorescence-based membrane potential assay was used to measure ligand-induced hyperpolarisation. Data was fitted to the operational model in GraphPad Prism to calculate ligand efficacy.

Results. The concentrations of tetracycline needed to achieve maximal (0.1µg/ml) and submaximal (uninduced) responses for a high efficacy ligand were initially determined. We measured the efficacy of seven CB_2 ligands in these conditions (n = 3-7 per drug). The recently described CB_2 agonist AK-F-064 (Kallinen et al, 2023) exhibited the highest efficacy (τ= 10.6±1.7, ≥ CP55,940) and anandamide (AEA) the lowest (τ= 0.70±0.2).

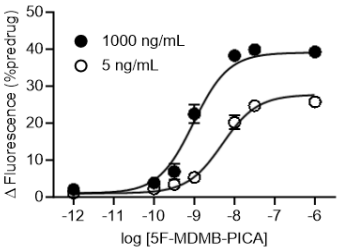
Discussion. This system can measure and differentiate the distinct efficacy of CB_2 ligands, something that has not been achieved before using the operational model. This system will also be useful in exploring the efficacy of CB_2 ligands at naturally occurring variants of the CB_2, to gain insight into whether these variants might affect the action of endogenous or exogenous ligands for CB_2.

Black J.W. and Leff P (1983) Proc R Soc Lond B Biol Sci, *220*(1219), pp.141-162
Kallinen A et al (2023) ACS Chem Neurosci 14(16):2902-2921

P634. A novel system for determining Cannabinoid Receptor 1 (CB_1) activity by employing the operational model.

Humayra Zaman[1], Marina Junqueira Santiago[1], Mark Connor[1]. Macquarie Medical School, Macquarie University[1], Sydney, NSW, Australia.

Introduction: CB_1 agonists are prescribed for pain, anxiety and depression. Important properties of therapeutic drugs include pharmacological parameters such as efficacy (τ) and functional affinity (K_A). τ and K_A can be determined by using the Black and Leff operational model (Black & Leff, 1983) to analyse concentration-response curves from tissue where receptor levels are different, with at least one condition where a maximal receptor occupancy produces a submaximal response. We have previously achieved this for CB_1 by use of an irreversible antagonist (Sachdev et al., 2019). Our new method uses the tetracycline-repressor (TREx) system to control receptor levels.



Aim: To determine the operational efficacy and functional affinity of CB_1 selective ligands using the TREx system.

Method: AtT20FlpnTREx cells stably expressing CB_1 were induced with various concentrations of tetracycline (Tet) to achieve different levels of receptor expression. The hyperpolarisation produced in response to K channel activation was measured by using a fluorescent membrane potential assay. Data was fitted to the Black and Leff operational model in GraphPad Prism to determine τ and K_A values.

Results: Preincubation with 1000 ng/mL Tet produced a maximal system response and 5 ng/mL produced a submaximal response by high efficacy CB_1 agonists. The τ value of Δ9-tetrahydrocannabinol in a system with spare receptors was significantly less than the high efficacy synthetic cannabinoid, 5F-MDMB-PICA, 0.58 ± 0.10 and 21.77± 3.92 (n=6, $P<0.05$) respectively. There was no correlation between operational efficacy and affinity.

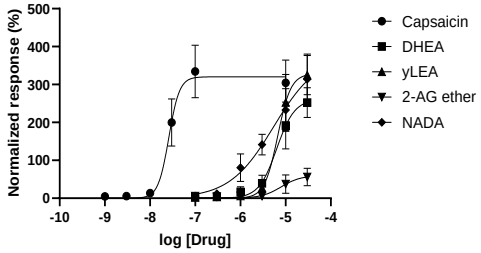
Discussion: This assay distinguished high and low efficacy agonists in a qualitatively similar way to experiments with the irreversible antagonist. Integration of the TREx system with the Black and Leff operational model is likely to be a valuable tool for measuring operational efficacy and functional affinity. This technique could be extended to other biological assays for the assessment of biased signalling and the screening of novel drugs targeting CB_1 receptors.

Black JW, Leff P (1983) Proc R Soc Lond B Biol Sci 220(1219):141-62
Sachdev S et al (2019) Br J Pharmacol 176(24):4653-65

P635. Endocannabinoid related derivatives of polyunsaturated fatty acids (PUFAs) activate TRPV1 ion channel.

Atnaf Abate[1,3], Marina Junqueira Santiago[1], Alf Garcia-Bennett[2,3], Mark Connor[1,3]. School of Medical Sciences, Macquarie University, Sydney, NSW, Australia[1]. School of Natural Sciences, Macquarie University, Sydney, NSW, Australia[2]. ARC Training Centre, *Facilitated Advancement of Australia's Bioactives* [3].

Introduction. PUFAs are essential for brain normal physiology, and they are utilized as supplements, with claims of enhancing brain health. Being precursors to endocannabinoids, the ligands derived from PUFAs may potentially interact with endocannabinoid system (ECS). While well-known endocannabinoids like 2-arachidonoylglycerol (2-AG) and anandamide (AEA) have been extensively researched, the activities of other endocannabinoids (γ-linolenoyl ethanolamide (GLEA), 2-arachidonyl glyceryl ether (2-AG ether), and N-docosahexaenoyl ethanolamine (DHEA)) at TRPV1 ion channel remain unclear.

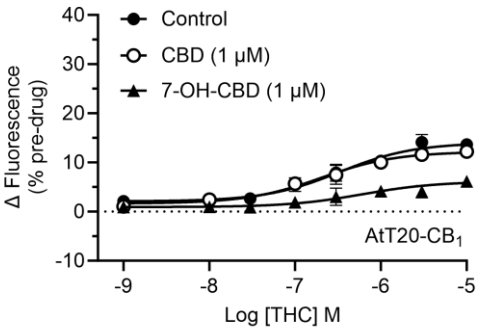


Aims. To examine the effect of the endocannabinoid and related metabolites of PUFAs on TRPV1 ion channel.
Methods. Human TRPV1-expressing HEK293 FlpIn/T-REx cell lines were studied by measuring changes in intracellular $[Ca^{2+}]i$ using a fluorescent dye.
Results. In HEK 293 FLIPIn/T-REx cells transfected with an empty vector; these drugs had no effect. However, in cells transfected with TRPV1, capsaicin, a typical TRPV1 activator, raised Ca-dye fluorescence significantly, reaching a maximum effect (E_{max}±SEM) of 304±60% above the pre-drug levels. The estimated potency (pEC_{50}±SEM) was 7.6±0.1. DHEA, GLEA and the PUFA-derived TRPV1 activator N-arachidonoyl dopamine (NADA) also activate TRPV1 ion channel with a notional pEC_{50} of 5.2±0.1, 5.1±0.1 and 5.3±0.5. At the highest concentration tested, the maximum effects of DHEA, GLEA and NADA (E_{max}±SEM) were 252±39%, 325±52%, 314±66% respectively. In contrast, 2-AG ether were minimally effective (pEC_{50} of 5.1±0.3, increasing Ca5 fluorescence by 56±23%).
Discussion. TRPV1 activation by these bioactive compounds could support brain health through endocannabinoid-mediated pain modulation.

P636. Modulation by CBD metabolite decreases THC signalling via CB_1 *in vitro*

Zoe Adam[1], Mark Connor[1], Marina Junqueira Santiago[1], [1]Macquarie Medical School, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, NSW, Australia.

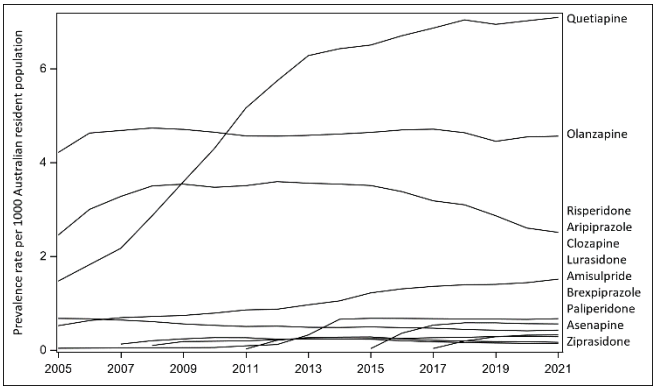
Introduction. Cannabidiol (CBD) is a phytocannabinoid used to treat seizure disorders and is part of a medication to treat spasticity and pain. There is limited information about the pharmacological activity of the metabolites of CBD and how they may contribute to, account for, or modulate the therapeutic effects of cannabinoids.
Aims. To examine the activity of CBD and its three major metabolites, 6α-hydroxy cannabidiol (6α-OH-CBD), 7-hydroxy cannabidiol (7-OH-CBD) and 7 carboxy cannabidiol (7-COOH-CBD), (up to 30 µM) on CB_1 signalling.



Methods. We used a fluorescence-based membrane potential assay (MPA) to measure K channel activation in AtT20-CB_1 cells. A bioluminescence-based assay was used to measure cAMP levels in HEK293-CB_1 cells. Maximum responses were compared with an ordinary one-way ANOVA with Dunnett's correction for multiple comparisons with $p>0.05$ considered significant.
Results. CBD metabolites did not activate CB_1 in either assay. At concentrations greater than 3 µM, CBD metabolites hyperpolarized both AtT20-CB_1 and wildtype AtT20 cells. In AtT20-CB_1 cells, preincubation with 7-OH-CBD (1 µM) inhibited the response to Δ^9-tetrahydrocannabinol (THC, 10 µM) (THC 14±2, THC+7-OH-CBD 6±1, $p<0.05$, n=4-11), this inhibition was greater than that produced by CBD (1 µM) (THC+CBD 12±1, $P<0.05$). 6α-OH-CBD and 7-COOH-CBD did not modulate the effects of THC. None of the metabolites significantly affected the response at native SST receptors.
Discussion. This study suggests that 7-OH-CBD may have pharmacologically significant effects at CB_1 at concentrations relevant to therapeutic dosing. Further, the pharmacodynamic interactions between CBD and THC due to inhibition of THC agonist activity by this metabolite may be more complex that just the direct activity at CB_1 owing to its metabolism by differentially expressed P450 enzymes that are not shared by 6α-OH-CBD and 7-COOH-CBD.

P637. Real-world evidence of antipsychotic utilization in Australia (2000–2021) using two datasets

Ramya Padmavathy Radha Krishnan[1], Christopher Harrison[1,2], Jacques Raubenheimer[1], Nicholas Buckley[1]. Biomedical Informatics and Digital Health, Univ of Sydney[1], NSW, Australia; Menzies Centre for Health Policy and Economics, Univ of Sydney[2], Sydney, NSW, Australia.



Introduction. Antipsychotic utilization is increasing globally, with significant off-label prescribing.
Aims. To determine antipsychotic utilization patterns in Australian adults, with a focus on on-label and off-label prescriptions.
Methods. We summarized trends in antipsychotic usage from PBS (Pharmaceutical Benefits Scheme) 10% dataset containing patient-level information on medicines dispensed in Australia between 2005–2021. We analysed diagnostic data for antipsychotics from BEACH (Bettering the Evaluation And Care of Health), a cross-sectional national survey from 2000–2016 consisting of data from general practitioner-patient encounters.
Results. We observed steady increases in both incidence and prevalence of antipsychotics, with an annual growth rate of 6.6%, mainly attributed to second-generation antipsychotics. As shown in the figure, quetiapine, olanzapine and risperidone were the most commonly prescribed. Among the patients receiving quetiapine, 35% were given the 25mg low dose without titration, with a median treatment duration of 85 (IQR 84–193) days. Analysis of diagnostic indications from BEACH indicated that 27% of antipsychotic prescriptions were off-label for indications such as depression, dementia, anxiety and insomnia, at much lower prescribed daily dosages.
Discussion. Each dataset adds a unique perspective to the concerning trend of increased antipsychotic utilization in Australia, with a significant proportion of off-label use. This could have a cascading effect on the development of adverse effects; more studies are required to understand the risks.

P638. Personalising selection of antifungals: can we predict lack of response to voriconazole?

Eman Wehbe[1], Thulashigan Sreeharan[1], Gaurav Sutrave[2], Indy Sandaradura[2,3], Jiin-Nyuk Fung[4], Jan-Willem Alffenaar[1] Sophie L Stocker[1]. School of Pharmacy, Faculty of Medicine & Health, Univ of Sydney[1], Sydney, NSW; Westmead Institute for Medical Research, Univ of Sydney[2], Sydney, NSW; Centre for Infectious Diseases and Microbiology, Westmead Hospital[3], Sydney, NSW; Dept of Pharmacy, Westmead Hospital[4], Sydney, NSW.

Introduction. Up to 40% of patients who receive the antifungal drug voriconazole are not exposed to therapeutic drug concentrations and 30% prematurely discontinue therapy due to adverse effects. This variability in response is primarily influenced by genetic variation in CYP2C19.
Aims. To investigate if CYP2C19 genotype can predict failure to achieve therapeutic serum levels of voriconazole and/or necessitating switch to alternative effective antifungal therapy. Secondary aims are to examine the relationship between CYP2C19 genotype, voriconazole exposure, and incidence of adverse effects.
Methods. An observational study of patients administered voriconazole (1/05/2019 – 31/05/2022) at Westmead Hospital. Data on voriconazole administration (dosing history, plasma drug concentrations), drug-related adverse effects, and alternative antifungal use were obtained from electronic medical records. Buccal swabs were collected from patients to determine CYP2C19 genotype. Data analysis was conducted using GraphPad Prism 9.5.1.
Results. Of the 52 patients recruited to date, 29% (15/52) were CYP2C19 ultra/rapid metabolisers (URM), 29% (15/52) were normal metabolisers (NM) and 42% (22/52) were intermediate metabolisers (IM). An adverse effect was experienced by 53% (8/15) of URM, 80% (12/15) of NM and 68% (15/22) of IM. Mean (±SEM) trough voriconazole concentrations were 0.96±0.15 mg/L for URM, 1.41±0.32 mg/L for NM and 1.71±0.40 mg/L for IM ($p>0.05$). Overall, 42% (22/52) of patients were switched to an alternative antifungal. Of these patients, 27% (6/22) were URM, 23% (5/22) were NM and 50% (11/22) were IM.
Discussion. Patients were frequently switched from voriconazole to alternative antifungals due to adverse effects. CYP2C19 URM tended to have lower exposure to voriconazole and experience fewer adverse events. Recruitment of additional patients will help confirm these preliminary findings and identify factors associated with the requirement to switch to alternative antifungal therapy.

P639. Psychotropic use in older people with and without dementia in Australia.

Weisi Chen[1], Edward C.Y. Lau[1], Christine Y. Lu,[1] Edwin C.K. Tan[1]. School of Pharmacy, The University of Sydney[1], Sydney, NSW, Australia

Introduction. People with dementia are high users of psychotropic medications; however, national estimates of psychotropic prevalence in this population are lacking.
Aims. To investigate the prevalence of psychotropic use in older people with dementia compared to those without dementia in Australia.
Methods. This was a cross-sectional study using linked 2021 Australian Census and Pharmaceutical Benefits Scheme (PBS) data. People aged 65 years and over who completed the 2021 Census were included. Dementia status was defined based on self-reported dementia diagnosis in the 2021 Census and/or any dispensing of an anti-dementia medication from 1 Jan 2016 to 31 Dec 2021. Medications were classified according to the Anatomical Therapeutic Chemical (ATC) classification system, with psychotropics defined as ATC N. Prevalence of psychotropic use in 2021 was reported descriptively and chi-squared tests were used to compare psychotropic use between people with and without dementia. All statistical analyses were conducted using R.
Results. A total of 3,850,509 older people were included, of which 177,809 (4.6%) were living with dementia. Compared to those without dementia, people with dementia were older (median age: 84 [IQR: 78-89] vs 73 [69-79]) and the majority were female (59% vs 53%). People with dementia were more likely to be exposed to polypharmacy (81% vs 63%, p<0.001), and psychotropics (68% vs 42%, p<0.001), compared to those without dementia. Antipsychotics (18% vs 1.9%, p<0.001), antidepressants (46% vs 22%, p<0.001), antiepileptics (9.2% vs 2.7%, p<0.001) and benzodiazepines (16% vs 12%, p<0.001) were more commonly prescribed in people with dementia when compared to people without dementia. The prevalence of antidementia drug use was 31% in people with dementia with donepezil being the most commonly prescribed.
Discussion. Almost 70% of people with dementia use a psychotropic in Australia. As these medications are associated with an increased risk of adverse events, the risks and benefits of using psychotropics in people with dementia should be carefully balanced by clinicians.

P641. Poisonings in older adults with dementia: a systematic scoping review and meta-analysis.

Ilsa R. Wojt[1], Rose Cairns[1,2], Edward Chun Ye Lau[1], Edwin C.K. Tan[1,3] School of Pharmacy, Faculty of Medicine and Health, The University of Sydney[1], Sydney, NSW, Australia. NSW Poisons Information Centre, The Children's Hospital at Westmead[2], Sydney, NSW, Australia. Centre for Medicine Use and Safety, Monash University[3], Melbourne, VIC, Australia.

Introduction. Older people with dementia are highly susceptible to medication errors and poisonings yet remain high consumers of medications to manage their chronic comorbidities. Despite their increased risk for poisonings, few studies have investigated and described the incidence, risk factors, associated agents and outcomes of poisonings in this population.
Aims. This review aimed to describe the key agents, incidence, risk factors and disposition of poisonings in people with dementia reported within the literature.
Methods. This review followed the Joanna Briggs methodology for systematic scoping reviews. Medline, Embase, PsycINFO and CINAHL databases were searched for articles from 1st September 2001 to 1st September 2021 that reported on poisonings in people with dementia diagnoses. Two authors independently assessed articles for eligibility and extracted relevant data to answer our aims. A meta-analysis of the incidence of poisonings in people with dementia across applicable studies was conducted.
Results. Of 4,579 articles, 18 were included for final synthesis. Nervous system medications were implicated in over half of all medicinal poisonings, with anti-dementia agents, benzodiazepines and opioids as the most common classes. The annual incidence of poisoning varied across definitions from 3% for ICD-defined poisonings to 43% for ADE-defined poisonings. Several risk factors were identified, including multimorbidity, psychotropic medication use and residing in residential care. Where described, up to one in five poisonings resulted in hospitalisation and in death.
Discussion. Poisonings are common in people with dementia, involving commonly prescribed medications or easily accessible substances. Given the significant outcomes associated, further research is required to better understand these poisonings and improve public health strategies to reduce the occurrence of this preventable harm.

P642. The availability of mobile applications to facilitate deprescribing: A scoping review

Lina Okati[1], Danijela Gnjidic[1], Sarita Lo[2,3], Susan Jiayu Li[2], and Janani Thillainadesan.[2,3,4] School of Pharmacy, University of Sydney[1], Department of Geriatric Medicine; Concord[2],Centre for Education and Research on Ageing, Sydney Local Health District[3],Concord Clinical School, University of Sydney[4]

Introduction. Deprescribing optimises medication use through the supervised withdrawal of medications that are no longer required and/or are causing more harm than benefit for patients. However, there are barriers to deprescribing such as lack of time to review medications, insufficient resources and inadequate training. Increasingly, mobile apps are being utilised by patients and healthcare professionals (HCPs) as an adaptable and personalised support tool for managing patients' medications.
Aims. The aim of this scoping review is to describe the availability and characteristics of mobile apps in the area of deprescribing.
Methods. Mobile apps were identified through searches on the Apple Store and Google Play Store, in August 2023 using the keywords "deprescribing", "polypharmacy", "drug reduction", and "medication reviewing". The title and description of the apps found were screened to assess eligibility for inclusion. All the included apps were then downloaded and features were recorded independently by two researchers. The quality of the included mobile apps was assessed using the Mobile App Rating Scale (MARS). The protocol for this study has been registered on OSF.
Results. In total 1058 apps were screened in the Apple Store and Google Play Store. Five deprescribing apps met the study inclusion criteria. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was utilised for the reporting of findings with reasons for exclusion also included. Preliminary findings include that these apps predominantly concentrated on educating patients and HCPs about deprescribing and how to apply it into clinical practice. Three apps targeted both patients and HCPs while the other two were specifically designed for HCPs, with one offering individualised recommendations for deprescribing through yes-or-no prompt questions allowing patient specific data to be entered.
Discussion. There are few mobile apps available to support deprescribing. The majority of deprescribing apps cater to enhancing knowledge among both patients and HCPs. This underscores the potential of mobile technology like mobile apps in bridging the knowledge gap and enhancing the practical application of deprescribing in clinical settings.

P643. Prevalence of and risk factors for drug-related readmissions in older adults

Narisha Prasad[1], Edward C.Y. Lau[1], Ilsa Wojt[1], Jonathan Penm[1,2], Zhaoli Dai-Keller[1,3], Edwin C.K. Tan[1,4]. School of Pharmacy, The University of Sydney[1], Sydney, NSW, Australia; Department of Pharmacy, Prince of Wales[2], Randwick, NSW, Australia; School of Population Health, The University of New South Wales[3], Sydney, NSW, Australia. Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia[4].

Introduction. Older adults are at risk of experiencing drug-related problems (DRPs), especially following hospital discharge. Drug-related readmissions are of particular concern given their burden on the patient and healthcare system. However, previous studies have reported inconsistent results on its prevalence and risk factors.
Aims. To assess the prevalence of drug-related readmissions in older adults and investigate the drug classes, preventability and risk factors most associated with these readmissions.
Methods. A systematic review was conducted. Four databases (MEDLINE, Embase, CINAHL and Scopus) were searched. Meta-analysis was performed to estimate the pooled prevalence of drug-related readmissions with further subgroup analyses to explore heterogeneity between studies.
Results. A total of 1,978 studies were identified in the initial search, of which four studies were included in the final synthesis. A pooled prevalence of 9% (95% CI 2%-8%) was found for all drug-related readmissions, and 6% (95%CI 4%-10%) for adverse drug reaction related readmissions. Overall, 15% to 22% of readmissions were deemed preventable. Polypharmacy was identified as a prominent risk factor, with anticoagulants, antibiotics, psychotropics and chemotherapy agents being the most associated drug classes. Comorbidities that were most associated with readmissions included cancer, liver disease, ischaemic heart disease and peptic ulcer disease.
Discussion. Almost 1 in 10 older adults discharged from hospital experienced a drug-related hospital readmission, with about one fifth of these deemed preventable. Several comorbidities, polypharmacy and the use of high-risk drugs were identified as prominent risk factors for readmission. Further research into causes of drug-related readmissions can assist in the development of effective medication management interventions.

P644. Six-country comparison of antimicrobial consumption in Latin American hospitals

G. H. Marin[1], L. Giangreco[1], Y. Hernández[1], C. Dorati[1], P. Mordujovich-Buschiazzo1, M. R. Bay[2], G. M. A. Gonzalez[2], F. Aldunate[3], M. López Peña[4], A. Martínez Parra[4], S. M. Ching Fung5, M. J. Alfonso Arvez[6,8], H. Mantilla Ponte7, D. Marin[8], R. Rojas[8], J. L. Castro[8]. [1]University Centre for Pharmacology (CUFAR). Faculty of Medical Sciences. National University of La Plata (UNLP). Argentina.[2]PharmacyService, HIGA San Roque, Gonnet, La Plata. Argentina. [3]Pontifical Catholic University (PUC). Chile.[4]Directorate of Medicines and Technologies in Health. Ministry of Health and Social Protection. Colombia. [5]Costa Rican social security (CCSS). Costa Rica. [6]National Health Surveillance Directorate (DINAVISA). Ministry of Public Health and Social Welfare. Paraguay. [7]General Directorate of Medicines, Supplies and Drugs (DIGEMID). Ministry of Health. Perú. [8] Pan-American Health Organization (PAHO). WDC, USA. [8]Centre for Medicine Use and Safety, Faculty of Pharmacy and Pharmaceutical Science, Monash University, Melbourne, VIC, Australia.

Introduction. Antimicrobial resistance (AMR) is a global public health challenge.
Aims. Describe antimicrobial consumption (AMC) in Latin American hospitals to inform local efforts in AMR prevention.
Methods. We applied WHO GLASS methodology using Defined Daily Doses (DDDs) and DDD/100 hospital discharges as measurement units. Antimicrobials were classified based on the WHO AWaRe (Access, Watch, Reserve) antibiotic book.
Results. Antibiotic consumption ranged from 182.48 - 2260.95 DDDs/100 hospital discharges. Qualitative analysis according to the AWaRe classification also showed a wide range in terms of consumption Access (ranging from 38.14% to 73.64%), Watch (ranging from 24.93% to 60.53%), and Reserve (ranging from 0.31% to 3.55%) groups expressed as a percentage of the total consumption.

	Consumption (DDD/100 hospital discharges)	Global analysis according to AWaRe classification (% overall global consumption)			
		Access	Watch	Reserve	Without classification
ARGENTINA (Hospital SR)	433.68	61.41	32.84	1.33	4.42
CHILE (Hospital NN)	182.48	38.14	58.31	3.55	0.0005
COLOMBIA (Hospital La Samaritana)	599.93	59.69	38.56	1.75	-
COSTA RICA (Hospital RCG)	1167.91	73.64	24.93	0.31	1.12
PARAGUAY (Hospital NI)	1771.61	38.66	60.53	0.82	-
PERU (Hospital DM)	2260.95	45.91	52.15	1.94	-

Discussion. There is considerable heterogeneity in antimicrobial selection across Latin American hospitals. Variation may be attributable to local resistance, prescription history, pharmaceutical marketing, healthcare education, and cultural practices, resulting in distinct consumption patterns among institutions.

P645. Consistencies between carbachol-induced contractions to clinical antimuscarinics in differently aged porcine bladders

Vineesha Veer[1], Russ Chess-Williams[1], Christian Moro[1]. Centre of Urology Research, Bond Univeristy[1], Robina, QLD, Australia.

Introduction. The first-line pharmaceutical therapies for managing overactive bladder (OAB) are antimuscarinics (Moro et al., 2011) where they inhibit spontaneous contractions during the filling phase by blocking the actions of acetylcholine. While clinically used antimuscarinics can inhibit contractions in juvenile models (Veer et al., 2023), it is unclear if they were as effective in older tissue samples. As the prevalence of OAB increases with age, it is likely that there may be receptor or functional alterations within the urinary bladder that could impact tissue responses to this drug class.
Aims. This study aims to find the differences in the ability of commonly prescribed antimuscarinics to inhibit contractions of the detrusor and compare these responses in juvenile and adult porcine tissues.
Methods. Strips of porcine detrusor from the adult or juvenile model were mounted in carbogen-gassed Krebs-bicarbonate solution at 37°C. The tissues were paired with carbachol concentration-response curves performed in the absence or presence of oxybutynin (1µM), solifenacin (1µM) darifenacin (100nM), tolterodine (1µM), trospium (100nM) and fesoterodine (100nM). Concentrations were chosen to ensure complete concentration-response curves in response to carbachol. pEC50 values for each curve were analysed and estimated affinities calculated. Ethical approval was not required for this study as tissues were sourced from the local abattoir after slaughter for the routine commercial provision of food.
Results. A right parallel shift was produced from the control in the juvenile detrusor for all antimuscarinics, with estimated affinities calculated for oxybutynin (7.47, n = 10) solifenacin (6.73, n = 8), darifenacin (7.58, n = 11), tolterodine (8.09, n = 8), trospium (8.69, n = 8) and fesoterodine (8.67, n = 8). A right parallel shift was produced from the control in the adult detrusor for all antimuscarinics, with estimated affinities calculated for oxybutynin (7.44, n = 9) solifenacin (6.63, n = 8), darifenacin (7.95, n = 9), tolterodine (7.93, n = 8), trospium (9.30, n = 9) and fesoterodine (8.54, n = 8). Comparisons of estimated affinities for each antimuscarinic between juvenile and adult tissues revealed no differences in each tissue's functional response to the six antimuscarinics ($p > 0.05$).
Discussion. Although preliminary, with this study ongoing, there appears to be no significant differences between detrusor functional responses to antimuscarinics of differently aged porcine samples. Further supporting that these medications can assist in the treatment of OAB and lower urinary tract symptoms in the detrusor layer.

Differences in compliance may be due to lifestyle or behavioural changes with age rather than alterations in the tissues ability to respond to the prescribed medication themselves.

Moro, C., Uchiyama, J., & Chess-Williams, R. (2011). Urothelial/lamina propria spontaneous activity and the role of M3 muscarinic receptors in mediating rate responses to stretch and carbachol. Urology, 78(6), 1442.e1449-1415.
Suskind, A. M. (2017). The Aging Overactive Bladder: A Review of Aging-Related Changes from the Brain to the Bladder. Curr Bladder Dysfunct Rep, 12(1), 42-47.
Veer, Vineesha, Chess-William, Russ, & Moro, Christian. (2023). Antimuscarinic actions on bladder urothelium and lamina propria contractions are similar to those observed in detrusor smooth muscle preparations. Neurourol. Urodyn.

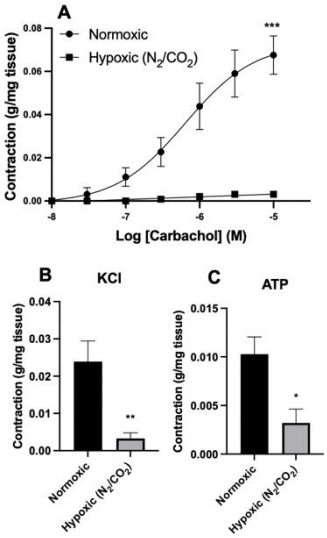
P646. 5-HT_3 receptors moonlight in mitochondria over their steady job at plasma membrane.

Santosh T R B Rao [1], Ilona Turek [1], Julian Ratcliffe [1 2], Simone Beckham [3], Cassandra Cianciarulo [1 2], Siti S B M Y Adil [1], Christine Kettle [1], Donna R Whelan [1], Helen R Irving [1] La Trobe Institute for Molecular Science, La Trobe University[1], P.O. Box 199, Bendigo, VIC 3552, Australia; Bio Imaging Platform, La Trobe University[2], Kingsbury Dr, Bundoora, VIC 3086, Australia; Regional Science Operations[3], La Trobe University, P.O. Box 199, Bendigo, VIC 3552, Australia.

Introduction. The 5-hydroxytrptamine 3 (5-HT_3) receptor is a member of the 'Cys-loop' family and the only pentameric ligand gated ion channel among the serotonin receptors. 5-HT_3 receptors play an important role in controlling growth, development, and behaviour in animals. Several 5-HT_3 receptor antagonists are used therapeutically principally in treating nausea and emesis but also for irritable bowel syndrome. Humans express five different subunits (A-E) enabling a variety of heteromeric receptors to form but all contain 5HT3A subunits. 5-HT_3 receptors are well known components of the plasma membrane.
Aims. To uncover the location and influence of the 5-HT_3 receptors on mitochondria.
Methods. 5-HT_3 receptor subunit protein sequences were computationally analysed for mitochondria target peptide signals. Epitope and fluorescent protein-tagged subunits were transiently transfected into HEK293T cells. Fluorescence and transmission electron microscopy were performed to track subunit location in subcellular compartments. Membrane potential and oxygen consumption assays were performed to analyse 5-HT_3 receptor influence on isolated mitochondria or in whole cells.
Results. Fluorescence microscopy and cell fractionation indicated that mitochondria contained both A and E subunits. Transmission electron microscopy revealed the subunits in the mitochondrial inner membrane, where they could form heteromeric complexes. The presence of A and E subunits altered membrane potential and mitochondrial oxygen consumption rates in whole cells and isolated mitochondria upon exposure to serotonin; this was inhibited by pre-treatment with ondansetron.
Discussion. It is likely that the 5-HT_3 receptors present on mitochondria have a moonlighting role as potential inducible mitochondrial uncouplers influencing cellular responses. It remains to be determined if mitochondrial location of these subunits contributes to variance in responses to 5-HT_3 receptor ligands and how the E subunit which has marked differential expression in the gut further influences such effects.

P647. Contractile response of the urothelium/lamina propria depressed by acute hypoxia *in vitro*

Elouise S Tye[1], Catherine McDermott[1], Russ Chess-Williams[1] & Donna J Sellers.[1] Centre for Urology Research, Faculty of Health Sciences and Medicine, Bond University, Gold Coast, QLD, Australia[1].

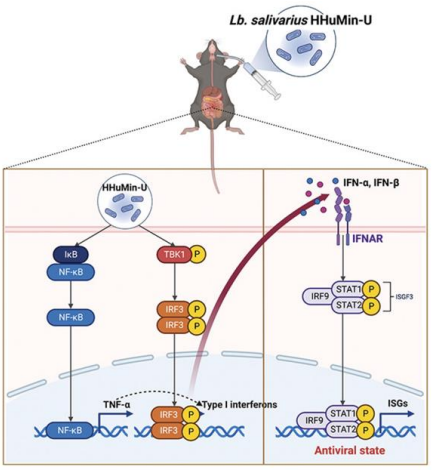


Introduction. Lower urinary tract dysfunction is associated with a reduced bladder blood flow and resultant hypoxia (Yamaguchi et al, 2014). The bladder mucosa which contains the urothelium and lamina propria is an important regulator of bladder function, but the consequences of hypoxia on mucosal function remains uncertain.
Aims. This study aimed to investigate the effects of acute hypoxia on contractile responses of the bladder mucosa *in vitro*.
Methods. The responses of isolated bladder mucosal strips from female pigs were examined, with tissues set up in Krebs bicarbonate solution gassed with either 5% CO_2 in O_2 (normoxic), or 5% CO_2 in nitrogen or 5% CO_2 in air.
Results. N_2/CO_2 induced hypoxia significantly decreased maximum contractile responses of the mucosa to carbachol to 5.0±1.5% of the normoxic (control) response ($P<0.001$, n=6), with the responses to ATP ($P<0.05$, n=5) and KCl ($P<0.01$, n=5) reduced to 32±16% and 14±3.8% of control respectively. The relaxation responses to isoprenaline were similarly attenuated. However, the potency of carbachol was not affected by hypoxia (-LogEC_{50} control 6.19±0.25 vs hypoxia 6.21±0.35). Preliminary results indicate that air/CO_2 also reduced contractile responses, although to a lesser extent than N_2/CO_2, with the maximal response to carbachol 52±8.2% of the normoxic control response (n=4).
Discussion. The results demonstrate a depressant effect of acute hypoxia on relaxation as well as contractile responses of the bladder urothelium. These changes may contribute to the bladder dysfunction associated with reduced blood flow and hypoxia.

Yamaguchi O et al (2014) Neurourology and Urodynamics 33:54-58.

P648. Lactobacillus salivarius HHunMin-U enhances the innate immune response against Norovirus infection

Jay-Young Jo[1], Da Hyun Kim[1], Minju Jeong[1], Jae Hwan Kim[1], Joe Eun Son[2], John J. Y. Lee[2]. Biotechnology, Yonsei University[1], SEL, South Korea; Program in Developmental and Stem Cell Biology, The Hospital for Sick Children[2], TOR, Canada.



Introduction. Norovirus is a highly contagious cause of gastroenteritis. Innate immunity, especially type I interferons, is vital in limiting its impact. A lactic acid bacterium called *Lactobacillus salivarius* HHuMin-U has been found to enhance intestinal immunity against norovirus by producing type I interferons, offering potential for future treatments.
Aims. To investigate the antiviral activity of *Lactibacillus salivarius* HHuMin-U toward norovirus and clarify the mechanism of it.
Methods. Effects of HHuMin-U treatment were evaluated with a murine macrophage cell line. The antiviral activity of HHuMin-U was tested in vitro by real-time PCR and ELISA. The transcriptomic changes after the treatment of the probiotics were analyzed by RNA sequencing and the results were validated with RT-PCR. Protein expression was measured by western blotting. Nuclear factor kappa B(NF-κB) and STAT2 nuclear translocation was detected by immunofluorescence. The in vivo effect of HhuMin-U was evaluated by oral administration of mice.
Results. HHuMin-U demonstrated significant suppression of murine norovirus replication and reduced viral RNA levels in macrophages. Transcriptome sequencing analysis (RNA sequencing) revealed that HHuMin-U markedly upregulated the expression of antiviral interferon-stimulated genes compared to the control treatment.
Discussion. Our findings highlight the species- and strain-specific importance of gut microbial composition in antiviral immune responses and suggest the potential use of HHuMin-U as a probiotic agent.

P649. The Functional Role of Phosphodiesterase Isoenzymes in the Isolated Porcine Urethra

Eriq Burovski[1], Iris Lim[1]. Faculty of Health Sciences and Medicine, Bond University[1], Gold Coast, QLD, Australia.

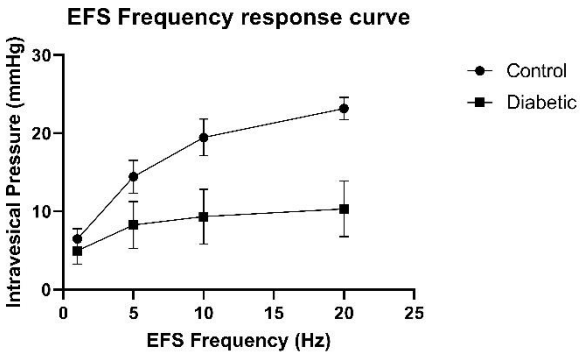
Introduction. Previous research has suggested a role for phosphodiesterase (PDE) isoenzymes in the control of the urethral smooth muscle contractility (Abrams et al., 2010; de Groat & Yoshimura, 2015; Rahardjo et al., 2021).
Aims. The present study aimed to investigate the role of PDE-4 and PDE-5 isoenzymes in the isolated porcine urethral smooth muscle and mucosal layers to identify potential targets for stress urinary incontinence management.
Methods. Using an organ bath setup, the effects of roflumilast (PDE-4) and sildenafil (PDE-5) (0.1 nmol/L – 10 µmol/L) on isolated porcine urethral mucosa-intact smooth muscle, denuded smooth muscle and mucosal layer were investigated. Unpaired Student's *t*-tests or a one-way ANOVA followed by a Dunnett's multiple comparisons tests was performed to identify statistically significant differences. A p-value of < 0.05 was considered statistically significant.
Results. The dose-dependent relaxation by roflumilast in urethral smooth muscle tissue strips with mucosa-intact was significantly enhanced compared to the denuded strips ($p<0.05$). Inversely, the relaxation induced by sildenafil was greater in denuded strips ($p<0.05$). In the presence of nitric oxide (NO) donor sodium nitroprusside (SNP), the attenuation effect of sildenafil in the denuded strips was enhanced ($p<0.05$). Sildenafil, in the presence of SNP, was more potent than roflumilast in attenuating the tonic contractions of the urethral smooth muscle strips (45% vs 24%). In the urethral mucosal strips, roflumilast (10 nmol/L and above) and sildenafil (1 µmol/L) significantly reduced the rate of spontaneous contraction ($p<0.05$).
Discussion. The results from the study suggest a potential role of the cAMP pathway in modulating spontaneous contractions within the mucosa, while the NO / cGMP pathway appears to be important in modulating urethral smooth muscle tonic contractions. Additionally, the findings also suggests that the presence of the mucosa may inhibit endogenous NO production. The complex interplay between the cAMP and cGMP pathways could be further investigated and identified as potential targets for SUI treatments.

Abrams P et al (2010) Neurourol Urodyn 29:213-240
De Groat WC & Yoshimura N (2015) Handb Clin Neurol 130:61-108
Rahardjo et al (2021) Res Rep Urol 13:139-145

P650. Comparison of diabetes-induced bladder dysfunction *in vitro* and *in vivo*

Aidan S McKeon, Catherine McDermott, Russ Chess-Williams, Donna J Sellers. Centre for Urology Research, Faculty of Health Sciences and Medicine, Bond University, Gold Coast, QLD, Australia.

Introduction. Bladder dysfunction affects a majority of patients with diabetes and undergoes a time-dependent change, exacerbated by poor glycaemic control, from the compensated to decompensated state.
Aims. We aimed to use *in vitro* and *in vivo* studies to investigate the mechanisms involved in diabetes-induced bladder dysfunction.
Methods. Diabetes was induced in female mice by treating with streptozotocin (50mg/kg ip daily for 5 days). Bladder function was examined 11 days later using isolated whole bladder preparations (WBP) and urethane-anaesthetised (0.9g/kg sc and 0.3g/kg ip) cystometry.



Results. Streptozotocin treatment increased ($P<0.05$) blood glucose from 9.3±0.4mmol/L (n=14) to 19.1±1.2mmol/L (n=15) and urine output was increased four-fold ($P<0.02$). In isolated whole bladders, increases in intravesical pressure in response to electrical field stimulation were significantly reduced in diabetic mice (see Figure). At 20Hz bladder responses from diabetic mice (10.3±3.6mmHg, n=4) were significantly lower ($P<0.03$) than controls (23.2±1.4mmHg, n=6). Similarly, pressure responses to the primary neurotransmitter adenosine triphosphate (ATP), were reduced ($P<0.04$) in diabetic mice (16.9±3.6mmHg) compared to controls (23.9±0.9mmHg). Also in vivo, cystometric experiments showed the peak pressure during voiding was reduced ($P=0.03$) in diabetic animals (28.16±0.89mmHg, n=11) compared with controls (32.40±1.63mmHg, n=8).
Discussion. The results suggest that short-term diabetes results in a reduced pressure development during voiding, which is caused by depressed bladder neurogenic contractions resulting from reduced bladder responses to the primary neurotransmitter ATP.