Annual Scientific Meeting
29th – 31st August 2018

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Abstract and Programme Booklet

Contents

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General Information

Welcome to the 2018 ASCEPT NZ meeting as part of Queenstown Research Week. On behalf of the organising committee we hope that you have an enjoyable, informative and educational meeting.

Organising Committee Members:

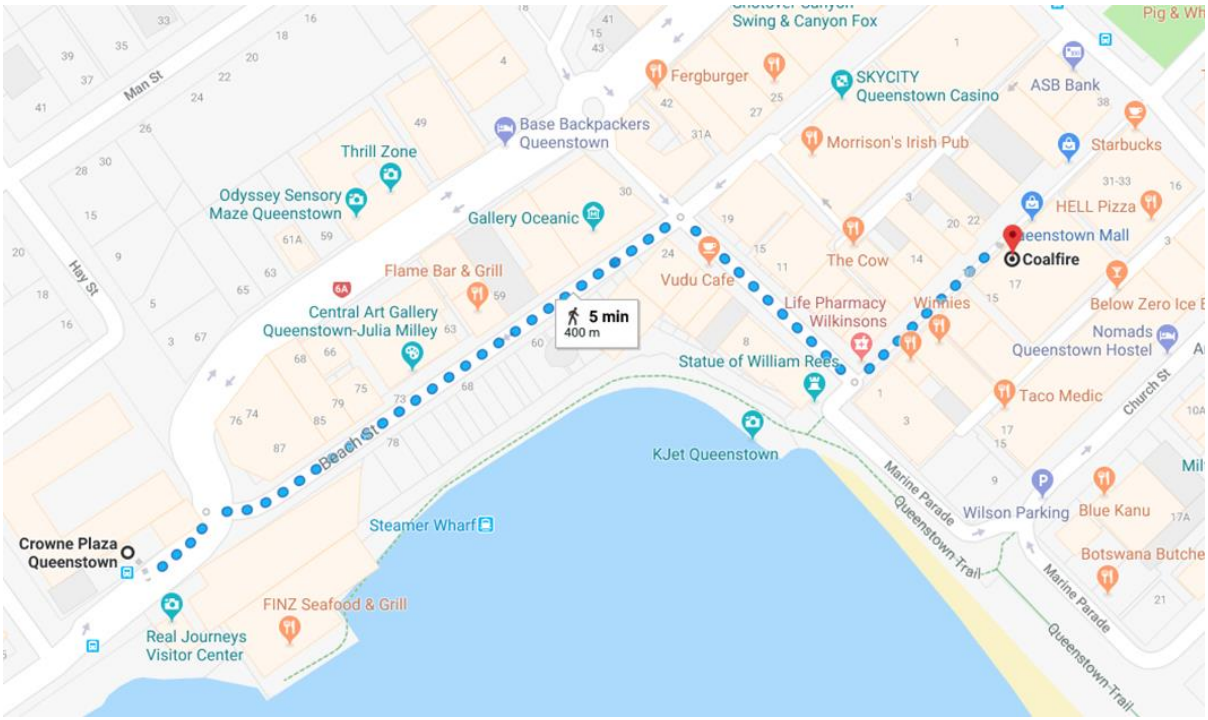
Nuala Helsby (chairperson) – University of Auckland
Hesham Al-Sallami (treasurer) – University of Otago, Dunedin
Chris Cameron (secretary) – Capital and Coast District Health Board
Katie Burns (conference convenor) – University of Auckland
Michelle Glass – University of Otago, Dunedin

Conference Venue:

Crowne Plaza Hotel
93 Beach Street
Queenstown

Conference Dinner:

Coalfire
Mall Street
Queenstown



Invited Speakers

Carl Kirkpatrick

Carl Kirkpatrick is the Professor of Pharmacy Practice and Director, Centre for Medicine Use and Safety, the Faculty of Pharmacy and Pharmaceutical Sciences, Monash University. His research focuses on optimising treatments for multi-drug resistant gram negative and gram positive bacteria, malaria, respiratory viruses and fungi across different at risk population groups via the application of quantitative clinical pharmacology, transmission modelling and anti-microbial stewardship methodologies. He has also applied these approaches to other therapeutic areas including cardiovascular, diabetes, cancer, and immunology. Carl has a growing portfolio of education research, with a special interest in the evaluation and remediation of employability skills in pharmacy students.

Catherine Sherwin

Dr. Catherine Sherwin is currently an Associate Professor (Tenured) and Chief of the Division of Clinical Pharmacology, Department of Pediatrics, University of Utah School of Medicine, and serves as Co-Director of the Pediatric Mentored Research Program, Director of the Clinical Pharmacology Fellowship Program, and Director of Pharmacometrics Utah Pharmacometrics Group. She earned her PhD in Paediatric Clinical Pharmacology at the University of Otago, Dunedin School of Medicine, New Zealand in 2007. Dr. Sherwin completed a one year Postdoctoral Fellowship at the University of Otago, Department of Women's and Children's Health and following that a two-year Postdoctoral T32 Fellowship training program in the Divisions of Clinical Pharmacology and Pediatric Rheumatology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio. Her pre-doctorate background includes a BSc(Hons) in Toxicology and Immunology and nursing within Cardiothoracic ICU/critical care. She received board certification from the American Board of Clinical Pharmacology in 2011. Catherine has been a member of American College of Clinical Pharmacology (ACCP) since 2005. She became a fellow of ACCP in 2014 and was recently elected to the Board of Regents. Dr. Sherwin is also a Senior Editor for the British Journal of Clinical Pharmacology.

Michelle Glass

Professor Michelle Glass is the Head of the Department of Pharmacology at the University of Otago. She is a molecular pharmacologist who studies the signalling of cannabinoid receptors. She is a Past- president of the International Cannabinoid Research Society (2015-2016) and has previously received their Young Investigator Award (2009) for her research on cannabinoid receptor signalling and function. She is the Chair of the Medical Cannabis Research Collaborative.

Mary Jane McCarthy

Mary Jane McCarthy manages ESR's Forensic Toxicology unit and Pharmaceutical Programme. She is a molecular pharmacologist by training having completed a PhD at University College London, and then worked extensively across the UK pharmaceutical sector. She also has medicines regulatory experience working at Medsafe and the Ministry of Health. While the majority of Mary Jane's research is focused around forensic toxicology, and the identification and surveillance of new psychoactive substances, she is also currently involved in developing ESR's capability in the area of medicinal cannabis testing and provision of regulatory expertise.

Karen Oldfield

Dr Karen Oldfield is a Medical Research Fellow at the Medical Research Institute of New Zealand. Dr Oldfield is an academic general practitioner who is currently undertaking clinical research. She has an interest in medical cannabis and its therapeutic potential and has very recently commenced a PhD through Victoria University (Wellington) looking at the clinical use of cannabinoids in New Zealand. She is a member of the Medical Cannabis Research Collaborative (MCRC).

Jennifer Martin

Professor Jennifer Martin is a physician and clinical pharmacologist in Newcastle Australia. She is Director of the National Health and Medical Research Council Centre of Research Excellence in Cannabinoids. Her team is leading a number of cannabis pharmacology studies with a variety of patient groups, products and formulations and is leading a Statewide palliative care access program of research with these products. She collaborates with colleagues in New Zealand, Israel, Netherlands, Canada and Brazil in this work.

Conference Programme

DAY 1 – Wednesday 29th August 2018 – WELCOME FUNCTION	
18.30 – 18.35	**Welcome** Nuala Helsby *Chair of ASCEPT NZ*
18.35 – 19.30	**Registration opens.** Drinks, nibbles, and networking function

DAY 2 – Thursday 30th August 2018 – ANNUAL SCIENTIFIC MEETING	
08.30 – 09.00	**Registration opens.** Tea and coffee available
Welcome – Nuala Helsby	
Oral Communications 1 – Chair: John Ashton	
09.00 – 09.15	**Jack Flanagan (A1)** University of Auckland *Evidence for an allosteric drug binding site in the lipid kinase PI3Ka*
09.15 – 09.30	**Mei Zhang (A2)** University of Otago *Stability of 6-thioguanine in mesalazine enemas: a possible future proctitis treatment.*
09.30 – 09.45	**Maia Van Kan (A3)** University of Auckland *Assessment of a PCR-based assay to detect TP53 adducts after exposure to DNA alkylating agents*
09.45 – 10.00	**Christa Macdonald (A4)** University of Auckland *Cannabinoid receptor expression and signalling in primary glioblastoma multiforme (GBM) cultures*
10.00 – 10.15	**Paul Chin (A5)** University of Otago *Real-world dabigatran concentration monitoring - Christchurch experience*
10.15 – 10.45	**Morning tea**
Student Presentations 1 – Chair: Hesham Al-Sallami	
10.45 – 11.00	**Riya Biswas (A6)** Auckland University of Technology *Role of MRP2 in oxaliplatin transport and response in colorectal cancer cells*
11.00 – 11:15	**Xiao Zhu (A7)** University of Otago *A kinetic model for describing signalling profiles of the cannabinoid-1 receptor*

11.15 – 11.30	**Minghan Yong (A8)** University of Auckland *Measurement of Thymidylate Synthase: the intracellular target of 5-fluorouracil*
11.30 – 11.45	**Kieran Deane-Alder (A9)** University of Auckland *Circulating microRNA expression and clozapine-induced cardiac injury*
11.45 – 12.00	**Nensi Shrestha (A10)** University of Otago *MAPK/ERK pathway and ALK inhibition has synergistic cytotoxicity in crizotinib resistant EML4-ALK-positive lung cancer*
12.00 – 12.15	**Isabelle Kuan (A11)** University of Otago *Should we use metformin in patients with chronic renal impairment? A systematic review of published metformin associated lactic acidosis cases*
12.15 – 13.00	**Lunch**
Symposium – Medicinal Cannabis or Cannabis as a Medicine? – Chair: Michelle Glass	
13.00 – 13.10	**Michelle Glass (A12)** University of Otago *An Introduction to Cannabis as a Medicine*
13.10 – 13.40	**Mary Jane McCarthy (A13)** Institute of Environmental Science and Research *Medicinal cannabis: a regulatory perspective*
13.40 – 14.10	**Karen Oldfield (A14)** Medical Research Institute of New Zealand *Medical Cannabis: Knowledge, Attitudes and Expectations amongst Wellington based General Practitioners*
14.10 – 14.50	**Jennifer Martin (A15)** University of Newcastle *Medical cannabis shows predictable pharmacological behaviour*
14.50 – 15.10	**Panel Discussion**
15.10 – 15.40	**Afternoon tea**
Oral Communications 2 – Chair: Chris Cameron	
15.40 – 15.55	**John Ashton (A16)** University of Otago *Cytotoxicity of curcumin derivatives in ALK+ non-small cell lung cancer cells.*
15.55 – 16.10	**Katie Burns (A17)** University of Auckland *mRNA expression of candidate 5-fluorouracil transporter genes in primary buccal mucosal cells*
16.10 – 16.25	**David Finlay (A18)** University of Otago *Evidence for in vivo toxicity of synthetic cannabinoids in rodents*

16.25 – 16.40	**Soo Hee Jeong (A19)** University of Auckland *An adapted assay to quantify thymine and dihydrothymine in patient urine after a thymine dose*
16.40 – 16.55	**Paul Chin (A20)** University of Otago *A nationwide survey on therapeutic drug monitoring of anti-tumour necrosis factor agents for inflammatory bowel disease*
16.55 – 17.00	**Comfort Break**
17.00 – 18.00	**ASCEPT AGM**
19.00	**Conference dinner**

DAY 3 – Friday 31st August 2018 – ANNUAL SCIENTIFIC MEETING

Invited Speaker – Chair: Steve Duffull

09.00 – 09.30	**Catherine Sherwin (A21)** University of Utah *Developing Precision Medicine Approaches in Paediatric Populations*

Student Presentations 2 – Chair: Malcolm Tingle

09.30 – 09.45	**Piyush Bugde (A22)** Auckland University of Technology *Knock out of ABCC5 gene using the CRISPR-CAS9 system increases gemcitabine sensitivity in pancreatic cancer (PANC-1) cells*
09.45 – 10.00	**Abigail Bland (A23)** University of Otago *Metformin in Combination with Crizotinib as an Anti-Cancer Treatment for EML4-ALK+ Non-Small Cell Lung Cancer.*
10.00 – 10.15	**Sudeep Pradhan (A24)** University of Otago *Evaluation of a study design to test the Intact Nephron Hypothesis*
10.15 – 10.30	**Mayerni Mutiara Situmorang (A25)** University of Auckland *Characterization of Nucleobase Transport in Human Cells*
10.30 – 11.00	**Morning tea**

Student Presentations 3 – Chair: Katie Burns

11.00 – 11.15	**Jaydeep Sinha (A26)** University of Otago *Development of predictive model for lean liver volume, a potential scaler for in vitro-in vivo extrapolation of drug clearance*
11.15 – 11.30	**Jamie Manning (A27)** University of Otago *An evaluation of novel allosteric modulators of the type-1 cannabinoid receptor.*

11.30 – 11.45	**Jakeb Petersen (A28)** University of Otago *Antihelminthic benzimidazoles mebendazole and albendazole kill cancer cells via classical apoptosis*
11.45 – 12.00	**Stephanie Thwaite (A29)** University of Otago *Attenuation of ischaemia reperfusion injury in hypertrophic hearts with a novel carbon monoxide releasing molecule, oCOm-21.*
12.00 – 12.45	**Lunch**
ASCEPT Guest Speaker – Chair: Nuala Helsby	
12.45 – 13.15	**Carl Kirkpatrick (A30)** Monash University *Vancomycin dosing, target attainment, and optimisation in patients receiving high-flux haemodialysis*
Oral Communications 3 – Chair: Dan Wright	
13.15 – 13.30	**Stephen Jamieson (A31)** University of Auckland *Anticancer efficacy of evofosfamide in head and neck cancer tumour models*
13.30 – 13.45	**Nuala Helsby (A32)** University of Auckland *Prediction of patients with severe to life threatening fluoropyrimidine-induced gastrointestinal toxicity using the thymine challenge test*
13.45 – 14.00	**Chris Cameron (A33)** Capital and Coast District Health Board *Fits or falls – it's all in the genes/jeans*
14.00 – 14.15	**Mark McKeage (A34)** University of Auckland *Do concomitant medicines impede safe and effective personalised lung cancer treatment in NZ?*
14.15 – 14.30	**Matthew Doogue (A35)** University of Otago *Recording Adverse Drug Reactions in New Zealand*
14.30 – 15.00	**Conference Close and Prize Giving**

Abstracts

A1: Evidence for an allosteric drug binding site in the lipid kinase PI3Kα

Flanagan, J.U.[1,2,3], Gong, G.Q.[3,4], Masson, G.[5], Lee, W-J.[4], Dickson J.M.J.[3,6], Buchanan, C.M.[3,4], Kendall, J.D.[1,3], Rewcastle, G.W.[1,3], Denny, W.A.[1,3], Shepherd, P.R.[1,3,4], Williams, R.W.[5]
Auckland Cancer Society Research Centre, University of Auckland[1], Auckland, NZ; Department of Pharmacology, University of Auckland[1], NZ; Maurice Wilkins Centre for Biodiscovery, University of Auckland[1], Auckland, NZ; Molecular Medicine and Pathology, University of Auckland[1], Auckland, NZ; MRC Laboratory for Molecular Biology[2], Cambridge, United Kingdom; School of Biological Sciences, University of Auckland[1], Auckland, NZ.

Introduction. The class IA phosphatidylinositol-3 kinase PI3Kα, phosphorylates the membrane embedded phospholipid phosphatidylinositol-4,5-phosphate ($PI(4,5)P_2$) making $PI(3,4,5)P_3$, which is a secondary messenger that promotes the membrane localisation of proteins with specialist domains that recognize the phosphorylated inositol unit. To perform this reaction, PI3Kα needs to bind its ATP substrate and interface with the cells plasma membrane to find its $PI(4,5)P_2$ substrate. The gene encoding PI3Kα is one of the most mutated in cancer, and many of the mutations create hyper-activated enzymes. Based on this, there is a huge effort to find inhibitors that are selective for the PI3Kα enzyme. The current molecules all blocking the ATP binding site.
Aims. To investigate the effect of ATP blockers on PI3Kα membrane binding.
Methods. A forester resonance energy transfer (FRET) reporter system and biolayer interferometry were used to follow PI3Kα binding to mixed lipid liposomes. Molecular docking and synthetic chemistry were used to study drug binding, and mass spectrometry was used to analyse drug binding sites.
Results. We identified a molecule that had a dramatic effect on wild type PI3Kα membrane binding. In dissecting the function of some of the drug-ATP binding site interactions, we developed a reactive probe molecule that led to the unexpected identification of a new binding site outside of the active site.
Discussion. We hypothesise that this new site is responsible for the membrane binding effect via an allosteric mechanism. These new data provide evidence that some PI3K inhibitors have two modes of action and further exploration of this could be used to develop new types of PI3Kα inhibitor.

A2: Stability of 6-thioguanine in mesalazine enemas: a possible future proctitis treatment.

Mei Zhang[2], Pepijn WA Thomas[1], Murray L Barclay[1,2]. Dept. of Clinical Pharmacology, Christchurch Hospital[1], and Dept of Medicine, University of Otago[2], Christchurch, New Zealand

Introduction. Six-thioguanine (6-TG) has been used as off-label rescue treatment in patients suffering from inflammatory bowel disease (IBD). Intrarectal 6-TG has also been shown to give symptomatic and histological improvement in proctitis. A Dutch group has pilot-tested 6-TG tablets dissolved in mesalazine enemas, in patients suffering from therapy-resistant proctitis, resulting in significant disease improvement.
Aims. To assess the stability of 6-TG at different temperatures after addition to mesalazine enemas.
Methods. In a separate glass bottle one 6-TG 10 mg tablet was dissolved in 100 ml mesalazine (Pentasa) enema solution. Samples were taken directly after dissolution (triplicate aliquots 0.1mg 6-TG/mL). Recovery (mean and SD as percentages) of 6-TG was assessed from samples stored at 37°C, 22oC, 4°C and -20°C, for 8 hours, 7 days, 7 days and 5 weeks, respectively. Measurements were taken during storage either hourly (8 hours), daily (7 days) or weekly (5 weeks). 6-TG concentrations were analysed using a validated liquid chromatography-mass spectrometry method.
Results. 6-TG concentrations remained stable at 37°C for 8 hours (110.9 ± 3.5%), at 4°C for 7 days (99.7 ± 6.2) and at -20°C for 5 weeks (132.7 ± 30.3%). Progressive degradation was seen from 1 day at 22°C until day 7 (59.9 ± 4.6%). There was a large variation for some triplicates (SD >15 % 6/31 triplicate batches).
Discussion. At room temperature 6-TG is only stable for 24 hours in a Pentasa enema solution. The variation of triplicate measurements may be related to the solubility of 6-TG tablets in the Pentasa enema.

Notes

A3: Assessment of a PCR-based assay to detect *TP53* adducts after exposure to DNA alkylating agents

Maia van Kan[1], Kathryn Burns[1] Nuala Helsby[1], Molecular Medicine and Pathology, University of Auckland[1], NZ

Introduction. Cyclophosphamide is a DNA alkylating prodrug and pharmacogenetic variability in the bioactivation and of cyclophosphamide and detoxification of its active metabolite occurs. This results in substantial inter-individual variation in the number of cytotoxic interstrand crosslinks (ICLs) formed, which will influence response to treatment.

Aims. To assess the ability of a previously published polymerase chain reaction (PCR)-based method [Stefanou et al 2012] to detect ICLs within the *TP53* gene following exposure to the active metabolite of cyclophosphamide (4-OHCP).

Methods. Pooled gDNA isolated from PBMCs were exposed to a range of concentrations of melphalan (positive control) or 4-OHCP at 37°C. The extent of inhibition of DNA polymerase amplification of three *TP53* sequences (6.8, 1.6 and 0.6 kb) relative to untreated controls was then assessed in replicate experiments. Following agarose gel electrophoresis the relative intensity of each ethidium bromide stained amplicon was determined using ImageJ 1.50i software.

Results: The modified assay could reproducibly detect melphalan-induced ICLs in gDNA using 6.8 and 1.6 kb target sequences. Detection of melphalan adducts in gDNA was linear ($r^2 > 0.95$) over 0.1 - 0.9 µg/mL and concentrations >2 µg/mL resulted in complete inhibition of PCR. This assay could also reproducibly detect ICLs after exposure of gDNA to 4-OHCP, although this required longer incubations (20 h vs 1 h). This was linear over 0.2 - 15 µg/mL ($r^2 > 0.90$). However, in contrast to melphalan, complete inhibition of PCR amplification was not achieved, even at very high concentrations (525 µg/mL). The limit of detection using the 6.8 kb amplicon was 16 and 26 ICL/10^6 nucleotides for melphalan and 4-OHCP, respectively.

Discussion. This assay appears to be a relatively simple way to quantify ICLs formed by melphalan and 4-OHCP and further work is underway to assess whether this assay could be used as a pharmacodynamic biomarker in patients treated with these drugs.

Stefanou et al (2012) British Journal of Clinical Pharmacology. 74; 5: 842-53.

A4: Cannabinoid receptor expression and signalling in primary glioblastoma multiforme (GBM) cultures

Christa MacDonald[1], David Finlay[2], Wayne Joseph[3], Graeme Finlay[3], E Scott Graham[4], Michelle Glass[2]. Department of Pharmacology[1], Auckland Cancer Society Research Centre (ACSRC)[3], Department of Molecular Medicine[4], The University of Auckland, Auckland, New Zealand; Department of Pharmacology, University of Otago[2], Dunedin, New Zealand.

Introduction. Current evidence in the literature is conflicting as to the effect of cannabinoids on GBM.

Aims. Determine the expression and signalling of cannabinoid receptor in primary GBM cultures and the effect of the cannabinoids on cell viability.

Methods. GBM cultures were developed by the ACSRC from patient tumour resections. Receptor signalling was measured by pERK activation and cell viability by alamarBlue assays. Receptor expression was determined by PCR, immunocytochemistry (ICC) and flow cytometry.

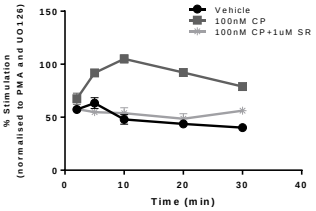


Figure: Timecourse of pERK detection in GBM culture NZB19 following treatment with 100nM CP55,940 +/- 1uM SR141716

Results. Variability in CB1 receptor expression was seen between the cultures. Expression correlated with the ability of cannabinoids to induce receptor signalling as detected by ERK phosphorylation. pERK assays in some cultures show a peak at 10 minutes when treated with 100nM CP55,940, an effect reversed in the presence of 1uM SR141716 (see figure), confirming active receptor. Concentration response curves in responding cells at 10 minutes with CP55,940, ACEA and THC produce EC50s in the nanomolar range. Cannabinoid effects on viability are highly varied between ligands and cell lines and significant effects are only seen in the micromolar range, with some ligands inducing a decrease in cell viability and others resulting in cell proliferation.

Discussion. Results show evidence for variation of CB1 expression between primary GBM cultures. CB1 receptors are functional and signal in the presence of nanomolar concentrations of cannabinoids but these concentrations do not appear to induce changes in cell viability and proliferation. A significant change in viability and proliferation is seen with cannabinoid treatments at micromolar concentrations only, suggesting a non-receptor mediated mechanism that is still being explored.

Cridge BJ and Rosengren RJ (2013) Cancer Manag Res, 5:301-13.

Notes

A5: Real-world dabigatran concentration monitoring - Christchurch experience

Chin P.K.L.[1,2], Lee M.H.[1], Smith M.[3], Doogue M.P.[1,2]
[1]Department of Medicine, University of Otago, Christchurch, NZ, [2]Department of Clinical Pharmacology, Christchurch Hospital, Christchurch, NZ, [3]Haematology Department, Christchurch Hospital, Christchurch, NZ.

Introduction. Dabigatran etexilate is an oral anticoagulant administered at fixed dose, stratified by renal function, with limited clinical monitoring. There is increasing recognition of the utility of using plasma concentrations of dabigatran, the active metabolite, to inform treatment decisions. However, there is limited information about real-world use of this assay. An in-house LC-MSMS dabigatran assay was recently made available clinically at Christchurch Hospital, New Zealand, with samples analysed as a weekly batch.
Aims. To describe the clinical reasons for measuring dabigatran concentrations and subsequent dabigatran etexilate prescribing decisions at Christchurch Hospital.
Methods. Patients with dabigatran concentrations during 2017 were identified from the hospital's laboratory database. These patients' health records were reviewed and the dabigatran etexilate prescribing before and after measuring dabigatran concentrations was examined.
Results. There were 34 patients with 41 dabigatran concentrations. The median (range) age of patients was 73 years (<1 – 89). The predominant indications for anticoagulation were atrial fibrillation (17/34, 50%) and venous thromboembolism treatment (16/34, 47%). The most common reasons for measuring dabigatran concentrations were: post-thromboembolic events despite dabigatran (12/41, 29%), uncertainty about concentrations because of renal function and pharmacokinetic drug-drug interactions (9/41, 22%), and post change in dose (8/41, 20%). The median (range) dabigatran concentration was 73 microg/L (0 – 470), reference range 30 – 130 microg/L. After the dabigatran concentration was reported, patient dabigatran etexilate prescriptions changed in 11/41 (27%) including: cessation (6), dose reduction (3) and dose increase (2). Of the remainder, prescriptions were unchanged in 20/41 (49%), and the clinical records did not reveal the dabigatran etexilate prescribing decision in 10/41 (24%).
Discussion. Measurement of dabigatran concentrations in the clinical setting was associated with a subsequent change in dabigatran etexilate prescriptions immediately after 27% of these tests.

A6: Role of MRP2 in oxaliplatin transport and response in colorectal cancer cells

Riya Biswas[1], Fabrice Merien[1,2], Jun Lu[1,4], Mark McKeage[3], Yan Li[1,4]. [1]School of Science, AUT, Auckland, NZ; [2]AUT-Roche Diagnostics Laboratory, Auckland, NZ; [3]Department of Pharmacology and Clinical Pharmacology, University of Auckland, Auckland, NZ; [4]School of Interprofessional Health Studies, AUT, Auckland, NZ.

Introduction. Oxaliplatin is important in the clinical treatment of colorectal cancer, but tumour resistance represents a critical therapeutic hurdle. MRP2 has been suggested to confer oxaliplatin resistance by pumping oxaliplatin out of cells. Previously, we have shown that silencing ABCC2, encoding MRP2, by small-interfering RNA (siRNA) increased cellular accumulation of platinum and sensitivity to oxaliplatin in HepG2 and PANC-1 cells.
Aims. The aim of this study was to mechanistically characterize the effects of oxaliplatin on MRP2 ATPase activities and to investigate the effects of silencing ABCC2 on oxaliplatin accumulation and sensitivity in human colorectal cancer Caco-2 cells.
Methods. Effects of oxaliplatin on MRP2 ATPase activities were measured by using plasma membrane suspension from sf9 cells overexpressing human MRP2. Caco-2 cells were transfected with scramble or ABCC2-siRNAs and then the cell surface expression of MRP2 protein was determined by flow cytometry and ABCC2 mRNA level measured by quantitative real-time PCR. Cytotoxicity and platinum accumulation was assessed by an MTT cell viability assay and ICP-MS (inductively coupled mass spectrometry), respectively.
Results. Oxaliplatin stimulated MRP2 ATPase activity is concentration dependent with an EC_{50} value of 8.3 ± 0.7 μM. The ABCC2 mRNA level and the cell surface MRP2 expression were significantly decreased after siRNA transfection. The IC_{50} values against oxaliplatin were significantly lower in Caco-2 cells transfected with three ABCC2-siRNAs (7.7 ± 0.1 μM ($P<0.05$), 8.4 ± 0.2 μM ($P<0.05$) and 7.0 ± 0.9 μM ($P<0.05$)) compared with those transfected with negative control (13.8 ± 1.5 μM). Silencing ABCC2 resulted in a 2-fold increase in the platinum accumulation in Caco-2 cells.
Discussion. In conclusion, our ATPase assay further confirmed that oxaliplatin is an MRP2 substrate. MRP2 limits oxaliplatin accumulation and response in human colorectal cancer Caco-2 cells. Screening tumour MRP2 expression levels, to select patients for treatment with oxaliplatin alone or in combination with MRP2 modulation, could improve outcomes of colorectal cancer treatment.

Notes

A7: A kinetic model for describing signalling profiles of the cannabinoid-1 receptor

Xiao Zhu[1], David B Finlay[2], Michelle Glass[2], Stephen B Duffull[1]. [1]School of Pharmacy, University of Otago, Dunedin, NZ, [2]Department of Pharmacology and Toxicology, University of Otago, Dunedin, NZ

Introduction. The term biased agonism is used to define a ligand that can act on a target receptor to differentially activate multiple signalling pathways[1]. Quantification of ligand bias is critical to lead compound optimisation. Signalling is affected by rapid ligand-mediated receptor internalisation. Hence, equilibrium models are not applicable as receptor number varies with time. A joint kinetic model is required to quantitatively assess the time-dependent modulation of three pathways (pERK, forskolin-induced cAMP signalling, and internalisation) of the cannabinoid-1 (CB1) receptor by six ligands (CP [reference ligand], WIN, anandamide, 2AG, THC and BAY).
Aims. (1) To develop a kinetic model that describes three signalling pathways coupled to the CB1 receptor, (2) to visualise bias profiles of the CB1 ligands.
Methods. A joint mechanism-based stimulus response model was developed. Internalisation was described by a target-mediated drug disposition model with a quasi-steady state assumption. pERK and cAMP were both described by a drug-effects model linked to the constitutive activity of the pathway. Ligand bias was calculated by ligand specific parameters (*i.e.,* ligand-mediated internalisation rate constant and ligand intrinsic efficacies for cAMP and pERK). Each parameter was (1) normalised to the reference ligand CP, and then (2) further normalised to a reference pathway (internalisation). The ligand bias profiles were visualised in a radar plot.
Results. The developed model adequately described the signalling profiles of the CB1 receptor. All model parameters were precisely estimated (<50% relative standard error) and were in agreement with prior values from the literature. From visualisation of the ligand bias profiles, two biased ligands (WIN and 2AG) were identified where both displayed higher selectivity towards cAMP pathway.
Discussion. This is the first report of a full analysis of this system under non-equilibrium conditions. The kinetic model approach enables quantification of ligand bias that may facilitate lead compound identification.

1. Urban, J. D., et al. (2007). J Pharmacol Exp Ther 320(1): 1-13.

A8: Measurement of Thymidylate Synthase: the intracellular target for 5-fluorouracil

Minghan Yong[1], Claire Bonnet[1], Adeli Lim[1], Soo Hee Jeong[1], Malcolm Tingle[2], Nuala Helsby[1].
Molecular Medicine and Pathology[1], Pharmacology and Clinical Pharmacology[2], University of Auckland, NZ

Introduction. Thymidylate synthase (TS) is the intracellular target for 5-fluorouracil and covalent inhibition of this enzyme is key for its action as a cytotoxic drug. Individuals with high protein expression are expected to be more resistant to the cytotoxic effects of this drug. Genetic polymorphism in the gene (*TYMS*) is reported to decrease transcription. Recent studies suggest that protein phosphorylation may affect both catalytic activity and gene transcription [1,2]. Hence, reliance on protein expression alone to predict which individuals are resistant to the cytotoxic effects of this drug may be flawed.
Aims. To assess the relationships between genotype, protein expression and phosphorylation status of TS.
Methods. HCT116, CaCo2 and HepG2 cells, plus human liver samples (n=4) were assessed. *TYMS* genotype was determined using RFLP-PCR [3]. TS protein expression was determined by PAGE-immunoblotting with anti-TS mAb. Human recombinant protein (rTS) was used as a reference standard. Incubation of 13.5 x 10^6 cells with 120 µM ^{3H}FdUMP (135 µCi), 3 h at 37°C, covalent binding was determined using liquid scintillation counting.
Results. *TYMS* genotype (3RG carrier status) had a small but significant ($P < 0.01$) effect on TS protein expression in human liver. A single immunoreactive band was detected, although the MW was approximately 3 kDa higher than the reference rTS protein. In contrast, neoplastic cells expressed a different profile of anti-TS immunoreactive bands. This included a protein with identical electrophoretic migration as rTS, as well as numerous other bands, suggestive of splice variants and dimers. TS protein appeared to be lower in CaCo2 than in HCT116 cells, neither of these cell lines are carriers of the 3RG genotype. Covalent binding of ^{3H}FdUMP was extremely low (2.2 ± 1.2 fmol/h/10^6cells) and in replicate experiments this was not substantially different between the three cell lines assessed.
Discussion. Assessment of the phosphorylation status of the TS protein in cells and human liver is ongoing.

1. Chen et al (2017) Journal of Biological Chemistry, 292: 13449–13458
2. Ludwiczak et al (2016) Molecular BioSystems, 12: 1333–1341
3. Mandola et al (2003) Cancer Research, 63: 2898–2904.

Notes

A9: Circulating microRNA expression and clozapine-induced cardiac injury

Kieran D Deane-Alder[1], Kathryn E Burns[2], Brandi L Bellissima, Malcolm D Tingle[1]. Department of Pharmacology and Clinical Pharmacology[1], Molecular Medicine and Pathology[2], University of Auckland, Auckland, New Zealand.

Introduction. Clozapine-induced myocarditis (CIM) is a rare but serious adverse event with an unusually high incidence in Australia and New Zealand (1). Diagnosis and treatment of CIM is often delayed, worsening outcomes, as symptoms are non-specific (2). The mechanism of cardiotoxicity is not well-understood, and may also lead to cardiomyopathy. Circulating microRNA (miRNA) offer an untapped resource that may elucidate the presence and mechanism of these adverse events.

Aims. To identify miRNA in patient plasma that could serve as potential biomarkers of clozapine-induced myocarditis or cardiomyopathy and contribute to understanding the mechanism of cardiotoxicity.

Methods. MicroRNA expression in the plasma of patients taking clozapine (CLZ) who had drug-induced myocarditis (n = 4), drug-induced cardiomyopathy (n = 3) and those who had neither (n = 12) was interrogated by microarray. miRNA that were highly-expressed in the microarray and identified in the literature as involved in cardiac pathology were investigated further. These were assayed by qRT-PCR in the same patients and compared to healthy controls (n = 4).

Results. Circulating microRNA were not significantly differentially expressed in patients who had myocarditis or cardiomyopathy. The qRT-PCR assay of cardiac-associated miRNA revealed that patients taking CLZ had 10-fold increased expression of let-7b ($p < 0.001$) compared to healthy controls. miR-1, -486, -106b, -122, -22, -23a, -142, -17, -133a and -221 were also differentially expressed in patients taking CLZ ($p < 0.05$).

Discussion. Our results suggest that circulating microRNA may not be predictors of clozapine-induced cardiac injury. In the literature, expression of let-7b and miR-486 has been associated with heart defects and injury, alongside miR-1, -106b, -122, and -133a (3,4). It is therefore possible that patients taking clozapine experience subclinical toxicity even if they do not suffer myocarditis: microRNA expression may not be selective simply because it is too sensitive.

1. Bellissima BL, (2018). PhD [thesis]. Auckland (New Zealand): The University of Auckland.
2. Bellissima BL, Tingle MD, Cicović A, Alawami M, Kenedi C, (2018). J Cardiol. 259:122–9.
3. Martínez-Micaelo N, Beltrán-Debón R, Baiges I, Faiges M, Alegret JM, (2017). J Transl Med. 15(1):76.
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A10: MAPK/ERK pathway and ALK inhibition has synergistic cytotoxicity in crizotinib resistant EML4-ALK-positive lung cancer

Nensi Shrestha, Mhairi Nimick, Rhonda J Rosengren, John C Ashton. Department of Pharmacology and Toxicology, University of Otago, Dunedin, Otago, New Zealand.

Introduction. Lung cancer is the leading cause of cancer deaths worldwide. Anaplastic lymphoma kinase (ALK)-positive lung cancer most commonly expresses EML4-ALK fusion protein. Crizotinib is the first-line treatment for ALK-positive lung cancer, providing increased progression free survival and overall response rate compared to chemotherapy. However, patients invariable develop acquired resistance, typically within the first year of therapy.

Aims. The aim of this study was to investigate the combined effect of ALK inhibitor, crizotinib, and MEK inhibitor, selumetinib, in crizotinib resistance ALK-positive lung cancer.

Methods. Sulforhodamine B assays were performed to determine the cytotoxicity of single and combined treatment of crizotinib and selumetinib in ELM4-ALK-positive (H3122), ALK-negative (A549) and crizotinib resistance ALK-positive (CR-H3122) cells. Chou-Talay analysis was performed to determine the type of interaction of the combination treatment. Endogenous expression of p-ALK and p-ERK was determined by western blotting. Cell cycle and apoptosis assay were performed to investigate the effect of combination treatment on cell cycle arrest and mode of cell death.

Results. Combination of crizotinib and selumetinib had significantly greater cytotoxicity, showing a synergistic effect compared to the single drug treatments in H3122 cells. The combination treatment had no synergistic effect on A549 cells, indicating that the synergistic cytotoxic effect was specific to ALK. The most prominent cytotoxic effect of the combination treatment was observed on CR-H3122 cells allowing the synergistic suppression of cell viability even at low drug concentrations. Endogenous protein expression of p-ALK and p-ERK was decreased by combination of crizotinib and selumetinib. Moreover, combination treatment lead to increased G1 arrest and apoptosis compared to single treatment in H3122 cells.

Discussion. Our results showed that combination of crizotinib and selumetinib has potent cytotoxic effects in crizotinib resistant ALK-positive lung cancer. The proposed combination treatment could be a promising therapy for acquired crizotinib resistant ALK-positive lung cancers.

Notes

A11: Should we use metformin in patients with chronic renal impairment? A systematic review of published metformin associated lactic acidosis cases

Isabelle HS Kuan[1], Stephen B Duffull[1], Ruth L Savage[2], Daniel FB Wright[1]. [1]School of Pharmacy, University of Otago, Dunedin, NZ, [2] New Zealand Pharmacovigilance Centre, Dunedin School of Medicine, University of Otago, Dunedin, NZ

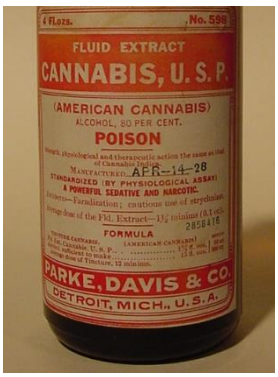
Introduction. Metformin has traditionally been contraindicated in patients with renal impairment because of a purported risk of metformin associated lactic acidosis (MALA).
Aim. To explore the relationship between chronic renal impairment, therapeutic doses of metformin, and the development of lactic acidosis in published case reports.
Methods. A systematic literature review was performed to identify cases of MALA. Intentional overdose cases were excluded. The WHO-UMC causality assessment tool[1] was used to assess the role of metformin in the development of lactic acidosis. The prescribed doses were compared to the Medsafe guideline for metformin renal dosing[2]. Evidence of sustained metformin plasma concentrations >5mg/L prior to admission was derived from data available in the case reports and from simulations conducted from a pharmacokinetic (PK) model[3].
Results. A total of 559 cases were identified of which 162 recorded pre-existing chronic renal impairment. Metformin was classed as a 'possible' cause in 89.5% of the renal cases. Nearly 50% of the cases had no other risk factor for lactic acidosis other than metformin use. The prescribed dose exceeded the Medsafe recommendation in 76% of the renal cases. After excluding n = 149 cases with acute renal impairment or no reported metformin plasma concentrations on admission, 10 of the 13 cases (76.9%) presented with metformin plasma concentrations >5mg/L. Simulations from a published PK model[3], using pre-admission creatinine clearance values, found that pre-admission steady-state plasma would have exceeded 5mg/L over much of the dosing interval in the majority of the cases.
Discussion. Metformin played a 'possible' role in nearly 90% of the cases of MALA with renal impairment. The majority of cases with renal impairment presented with metformin plasma concentrations >5mg/L, highlighting the need for a rational renal dosing guideline.

1. WHO-UMC [Available from: https://www.who-umc.org/media/2768/standardised-case-causality-assessment.pdf]
2. APOTEX NZ LTD (2008) [Available from: http://www.medsafe.govt.nz/profs/Datasheet/m/Metformintab.pdf]
3. Duong et al (2017) Eur J Clin Pharmcol 73: 981-990

A12: An Introduction to Cannabis as a Medicine

Michelle Glass, Department of Pharmacology and Toxicology, University of Otago City, Dunedin, New Zealand;

Introduction. Cannabis use has been reported now for thousands of years as a recreational agent and potential therapeutic. In the USA, cannabis appeared in the United States Pharmacopoeia for the first time in 1851. Federal restrictions on its use though began in 1937 and prohibition occurred with the Controlled Substances Act of 1970. A similar pathway was followed in a many developed nations. Since the legalisation of cannabis in California for medicinal purposes in 1996 however there has been a global push for recognition of "medicinal cannabis". This presentation will serve as an introduction to the symposium on Cannabis as a Medicine and will outline the commonly used terminology, and the current legal situation in New Zealand in order to provide a context for the talks which will follow.



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A13: Medicinal cannabis: a regulatory perspective

Mary Jane McCarthy, Forensic Toxicology and Pharmaceuticals, Institute of Environmental Science and Research, Wellington, New Zealand

Cannabis has been used for both recreational or medical purposes for centuries. Over the last few decades there has been increased scientific interest in identifying the active ingredients in cannabis, and investigating their pharmacological effects, particularly the cannabinoids THC and CBD. However, despite a wealth of anecdotal information about the medicinal effects of cannabis, there has been a limited number of well-designed clinical studies to support therapeutic claims and long-term safety, mostly due to production restrictions and government regulation. Public interest in the use of medicinal cannabis has heightened in New Zealand in the past couple of years. This follows a number of highly publicised cases of people applying for access to cannabis products for treating conditions such as epilepsy, multiple sclerosis and chronic pain.

Products containing cannabis are currently classified as controlled drugs in New Zealand, and Ministerial approval is required before products can be prescribed. At present there is limited availability of pharmaceutical grade cannabis-based products, and the associated costs are significant. The Ministry of Health is in the process of developing a regulatory scheme that will look to improve access to medicinal cannabis, and that will set quality standards around their manufacture and testing. The scheme will also enable domestic cultivation and manufacture.

This presentation will provide an overview of the current New Zealand medicinal cannabis regulatory environment, and provide a perspective on the proposed medicinal cannabis scheme, with a focus on mechanisms that would ensure product quality and safety.

A14: Medical Cannabis: Knowledge, Attitudes and Expectations amongst Wellington based General Practitioners

Dr Karen Oldfield[1,2], Dr Irene Braithwaite[1,2], Prof Richard Beasley[1,2], Dr Alex Semprini[1,2], on behalf of the Medical Cannabis Research Collaborative (MCRC). [1] Medical Research Institute of NZ, Wellington, NZ; [2] School of Biological Sciences, Victoria University of Wellington, Wellington, NZ.

Introduction. There has been increasing demand for 'medical marijuana' and its derivatives to be available in New Zealand. The New Zealand Health Survey of 2012/2013 reported that 42% of cannabis users had used cannabis for medicinal purposes in the 12 months prior (Ministry of Health, 2015), indicating that doctors and patients need to engage in discussion about the medical use of cannabis. This study is being undertaken to investigate the current state of knowledge around medical cannabis in New Zealand. This presentation describes a data subset from an ongoing study involving General Practitioners (GPs), Hospital Specialists and their patients.

Aims. To explore the current knowledge base of Wellington based GPs around medical cannabis and its uses, their attitudes to discussions about medical cannabis and how they would like educational content about medical cannabis to be delivered.

Methods. 40+ GPs in Wellington were contacted through peer and continuing medical education groups and invited to undertake a 7-page questionnaire about medical cannabis which covered the following topics; general knowledge, evidence in medical conditions, regulatory requirements, professional experience and demographic information. Data was then entered into the REDCap database for analysis using descriptive statistics to determine proportions with 95% Confidence Intervals.

Results. Currently undergoing analysis and will be presented at the ASCEPT conference in Queenstown.

Discussion. We hypothesise that knowledge of how to use and access medical cannabis will be limited in this Wellington-based GP cohort, as there is minimal educational content for the endocannabinoid system and medicinal cannabinoids at both undergraduate and post-graduate level. This will be discussed further at ASCEPT following the analysis of results.

Ministry of Health (2015) Cannabis Use 2012/13:New Zealand Health Survey. Wellington: Ministry of Health

Notes

A15: Medical cannabis shows predictable pharmacological behaviour

Jennifer H. Martin. School of Medicine and Public Health, University of Newcastle and Hunter New England Health District, NEW SOUTH WALES, Australia 2305.

Across many jurisdictions there is increasing interest in the use of plant and pharmaceutical cannabinoid products for both symptom management and treatment of disease. However, there are few registered products available for prescription. Even for those products registered, there is limited information available regarding their pharmacology across the myriad of disease states and populations in which patients and prescribers wish to use. Further compounding this problem is the state of pharmacological knowledge regarding non registered products. The dominant media narrative has been that cannabinoids are safe, have an individual and stable dose-response curve, and therefore standard pharmacological workup and early phase clinical trial work with different products across a number of disease states is not needed.
This presentation discusses the variety of chemical compounds including the cannabinoids known to contribute to the pharmacological and toxicological properties of cannabis. It discusses dose-concentration and concentration responses for the common cannabinoids and the effect of common drug-drug and drug-food interactions, and the need for more research, particularly in the population groups of interest. Toxicity is also commonly seen with cannabinoids, and the role of pharmacology in that aspect is also discussed. References to common drug interactions, role of the route of administration and variable and inconsistent effects of other drugs, diet and progressive disease on efficacy are discussed.

A16: Cytotoxicity of curcumin derivatives in ALK$^+$ non-small cell lung cancer cells.

Abigail R. Bland[1], Rebekah L. Bower[1], Rhonda J. Rosengren[1], John C. Ashton[1]. Department of Pharmacology and Toxicology, University of Otago[1], Dunedin, Otago, New Zealand.

Introduction. Non-small cell lung cancer with a primary ALK oncogene can be targeted effectively with ALK inhibitors such as crizotinib. However, cancer progress typically occurs within a year as drug resistance develops. One strategy to overcome drug resistance is to test the anti-cancer effects of novel cytotoxic agents in ALK lung cancer cells, and their ability to retain cytotoxicity in cancer cells that are ALK inhibitor resistant.
Aims. We therefore tested two novel cytotoxic agents; derivatives of curcumin, RL66 and RL118, in lung cancer cell lines.
Methods. The cytotoxicity of curcumin, RL66, and RL188 was tested alone and in combination with crizotinib in both ALK$^+$ lung cancer cells (H1322), crizotinib resistant ALK$^+$ cells (CR-H1322) and ALK$^-$ lung cancer cells (A549) using SRB assays. Western blot was used to assay for degree of ALK phosphorylation (hence activation) in response to the drugs.
Results. ALK$^+$ cells were 2-3x more sensitive to RL66 and RL118 than ALK$^-$ cells. This sensitivity was largely retained in crizotinib resistant cells. Combination of the curcumin derivatives with crizotinib provided results consistent with Bliss additivity. Crizotinib also inhibited phosphorylation of ALK, while the curcumin derivatives did not.
Discussion. ALK$^+$ lung cancer cells are sensitive to RL66 and RL188 cytotoxicity in the low micromolar range. The retention of this sensitivity in crizotinib resistant cells indicates that mechanisms of resistance to the two drug types are independent, with resistance to ALK inhibitors not necessarily causing cross-resistance to curcumin derivatives. Drug combination analysis further showed that the drugs combine in a way consistent with Bliss additivity, again indicating independent targets for crizotinib and the curcumin derivatives. Finally, Western blot showed that inhibition of ALK phosphorylation – the primary mechanism of crizotinib cytotoxicity – did not occur from exposure to curcumin derivatives, again indicating independent mechanisms of toxicity. These data indicate a potential combination therapeutic strategy, either by sequential administration of curcumin derivatives after the development of crizotinib resistance, or as an upfront combination

Notes

A17: mRNA expression of candidate 5-fluorouracil transporter genes in primary buccal mucosal cells

Kathryn E Burns[1], Janelle Moody[1], Daniel Allright[1], Nuala A Helsby[1]. Department of Molecular Medicine and Pathology, University of Auckland[1], Auckland, New Zealand.

Introduction. The use of 5-fluorouracil (5-FU) as a chemotherapeutic agent is often associated with severe (≥Grade 3) normal tissue toxicities, including oral mucositis (stomatitis). For many patients the underlying cause of these side effects is unknown. Additionally, the processes that mediate 5-FU transport are poorly understood in humans.
Aims. To investigate the expression of candidate drug transporters in primary buccal mucosal cells (BMC) collected from individuals who exhibit either high or low rates of 5-FU cellular uptake.
Methods. BMC collected from 24 healthy volunteers were incubated *ex vivo* with 5 µM [^{14}C] 5-FU for 5 min (37 °C and on ice) to determine the rate of drug uptake. BMC from individuals (n=4) with either high or low uptake (>10 or <2 pmol.min^{-1}.10^5 live cells^{-1}, respectively) were then pooled. The mRNA expression of 83 candidate transporter and 7 endogenous control genes in BMC was then determined by qRT-PCR in three replicate experiments.
Results. There was substantial inter-individual variability in the rate of 5-FU uptake into BMC, ranging from 0.1±0.01 to 15.3±3.3 pmol.min^{-1}.10^5 live cells^{-1} (mean ± SD; p<0.0001). The rate of 5-FU uptake in the pooled high and low samples was confirmed to be significantly different (p<0.05). Of the large panel of candidate transporter genes only twenty two (27%) were expressed in BMC. These included both efflux (e.g. *ABCA1, ABCG1*) and uptake (e.g. *SLC2A1, SLC15A1, SLCO3A1*) transporters.
Discussion. Differential expression of any of the 22 drug transporters detected in primary BMC could influence the inter-individual variation in rate of 5-FU uptake into these cells. Work is ongoing to identify which of the transporters might contribute to 5-FU uptake into buccal mucosa.

A18: Evidence for *in vivo* toxicity of synthetic cannabinoids in rodents

David B Finlay[1], Timothy W Lefever[2], Brian F Thomas[2], Jenny L Wiley[2], Michelle Glass[1]. Department of Pharmacology and Toxicology, University of Otago[1], Dunedin, New Zealand; RTI International[2], Research Triangle Park, NC, USA.

Introduction. So-called "synthetic cannabis" is reported to be responsible for deaths in New Zealand – 20 between January and mid-September 2017 alone[1]. However, this term is highly misleading as there is in fact no cannabis in these drug preparations; they consist of chemicals which are dissolved in solvents such as methanol or acetonitrile and sprayed onto dried plant materials. Widespread proliferation in NZ communities and the reported fatalities associated with these compounds makes understanding them an urgent public health issue, but little is currently known about these compounds – including why they are sometimes toxic to humans.
Aims. To perform a pilot study to investigate responses in mice (behavioural, physiological, and post-mortem gross pathology) to treatment with two prevalent synthetic cannabinoids.
Methods. Adult male ICR mice were injected IP with 1 mg/kg of the synthetic cannabinoids AMB-FUBINACA or 4-*cyano*-CUMYL-BUTINACA, or vehicle (7.8% polysorbate-80/92.2% saline). Additional to observations, at several time points heart rate was determined using ECGenie (Mouse Specifics, Inc) and its associated analysis platform; and temperatures were recorded by rectal temperature probe. Approximately 24 h after dosing, animals were sacrificed by CO_2 asphyxiation, and plasma collected by cardiac puncture. Heart/lungs were then dissected, washed in saline and photographed.
Results. With rapid onset (~5 min after dosing), traditional cannabinoid effects were observed (hypothermia, catalepsy). Additionally however, hyperstartle reactions and muscle spasms (consistent with CNS stimulation), and adverse cardiac responses (arrhythmia with bradycardia) were detected. Gross examination of dissected lungs suggested synthetic cannabinoid-induced pulmonary haemorrhage may also occur.
Discussion. Policymakers continue to debate increasing access to cannabinoids on the assumption that they cause minimal harm. These findings indicate that there is an urgent need to better understand the manifestations of the toxicity of cannabinoid agonists, and the mechanisms by which they occur.

[1] NZ Herald report, 15th September 2017. https://goo.gl/D1yWVE Retrieved 5th July 2018

Notes

A19: An adapted assay to quantify thymine and dihydrothymine in patient urine after a thymine dose

Soo Hee Jeong[1], Claire Bonnet[1], Jun Peng Yang[1], Nuala Helsby[1]. Molecular Medicine and Pathology, University of Auckland[1], Auckland, New Zealand.

Introduction. Dihydropyrimidine dehydrogenase (DPD) is responsible for >80% of the catabolism of 5-fluorouracil (5-FU) and thus deficiencies in its activity can lead to severe 5-FU toxicity. Several methods have been proposed to detect patients with reduced DPD activity but are not without limitations. Assessment of thymine (THY)/dihydrothymine (DHT) urine ratios following a THY dose has been proposed as a method to determine DPD activity in cancer patients and an LC-MS/MS (APCI mode) assay [Ni et al 2013] has been used to do this.
Aims. To validate an adapted analytical method that can simultaneously detect THY and its metabolite, DHT, in human urine to support ongoing clinical studies that aim to assess DPD activity in cancer patients. To measure THY/DHT urine ratios in cancer patients following a loading dose of THY (250 mg p.o.) using the adapted assay.
Methods. Method validation was carried out using a blank urine sample that had THY and DHT levels below the limit of detection. Analyses were conducted using a HPLC coupled with a single quadrupole mass spectrometer (LC-MS; APCI mode). Separations were performed using a C18 column (3 mm * 150 mm, 5 µm) and detection by selective ion monitoring (SIM; positive mode) for each mass ion was used: m/z 131 (THY-d_4), 127 (THY) and 129 (DHT). Mobile phase used were 0.1% formic acid in LC-grade water and 0.1% formic acid in acetonitrile. Cumulative urine (0 - 4 h) were collected following a loading dose of THY and THY/DHT ratios were measured in thirty seven patients.
Results. The limit of quantification for THY and DHT was 25 ng/mL. Calibration curves were linear (r^2 above 0.99) between 25 – 1000 ng/mL (cf. 2 – 500 ng/mL for the LC-MS/MS method). If sample analytes were outside of these values they were then reanalysed by reconstitution in a smaller volume of mobile phase and injected with a higher volume. Intra and inter assay CV were all below 15 % and accuracy within 9%. THY/DHT ratios measured in patient urine samples were between 0.26 - 19.61 and similar to the ranges previously reported using LC-MS/MS assay.
Discussion. Comparisons with data from the original LC-MS/MS assay confirmed that the adapted assay was suitable for use in clinical study.

Ni et al (2013) J Pharm Biomed Anal 78-79: 129-35

A20: A nationwide survey on therapeutic drug monitoring of anti-tumour necrosis factor agents for inflammatory bowel disease

Pepijn WA Thomas[1], Paul KL Chin[1,2], Matthew P Doogue[1,2], Murray L Barclay[1,2]. Dept of Clinical Pharmacology, Christchurch Hospital[1], and Dept of Medicine, University of Otago[2], Christchurch, New Zealand

Introduction. Routine therapeutic drug monitoring (TDM) during treatment with anti-tumour necrosis factor (anti-TNF) agents in inflammatory bowel disease (IBD) may increase treatment efficacy and cost-effectiveness, and reduce the risk of loss of response.
Aims. To assess the current use of anti-TNF agent TDM, including trough concentration and anti-drug antibodies, amongst gastroenterology practitioners in New Zealand.
Methods. A web-based survey with 29 questions delivered to gastroenterologists and advanced trainees in New Zealand, identified by the NZ Society of Gastroenterology.
Results. Response rate was 48% (48/100). Adalimumab (ADL) was the most common initial anti-TNF agent used (78%, infliximab 22%). Ninety-three percent used TDM (n=40), mainly in cases of non-response or loss or response (35/40). Most respondents (93% and 83% for ADL and IFX, respectively) measured concentrations within 24 hours prior to the next administration. In a patient in clinical remission on anti-TNF agents, but with endoscopic inflammation, 73% would measure drug concentrations. In the presence of antidrug antibodies, 45% would add an immunomodulator in patients with active disease and 48% would add an immunomodulator in patients in remission. With low trough concentrations, 78% would make no changes to treatment if the patient was in remission, and 78% would increase the dose if there was active disease.
Discussion. TDM is routinely used by IBD gastroenterology clinicians in New Zealand. However, interpretation of results and treatment decision-making is variable, suggesting more guidance is required.

Notes

A21: Developing Precision Medicine Approaches in Paediatric Populations

Catherine MT Sherwin[1], Kate M Job[1], Jonathan E Constance[1]. Division of Clinical Pharmacology, Department of Pediatrics, University of Utah School of Medicine[1], Salt Lake City, UT, USA.

Introduction. Precision Medicine is a phrase that infers that the right treatment is provided in the right amount at the right time for the right individual. The Human Genome Project revealed that the information in approximately 20,000 genes are 99.9% identical from one person to the next. The remaining difference (0.1%) defines each individuals uniqueness. These differences may influence an individuals susceptibility to certain illnesses or determine how an individual responds to a drug. In general, drugs are metabolized in the body by a group of enzymes; in some cases, active drugs are deactivated, or pro-drugs are activated.
Aims. To assess the trends in utilization of recommended genetic screening and the acceptance of pharmacogenomic screening in relation to the development of precision medicine approaches in paediatric populations.
Results. Drugs that are safe for one individual may not be safe for another due to the differences in intra-individual metabolism; children can be even more sensitive than adults due to the maturation of these different enzymes. Clinical testing for genetic variation that may identify why a patient has a different response to a drug compared to other patients is referred to as pharmacogenomics. The genetic differences within metabolic pathways are responsible for individual variability in drug exposure and response. Pharmacogenetic testing provides an opportunity to optimize drug therapy for patients based on their specific genetic makeup.
Discussion. The U.S. Food and Drug Administration (FDA) has indicated pharmacogenomic testing is recommended for a variety of specific drugs and specific genetic biomarker testing has been incorporated into those drug labels. However, limited data exist in paediatrics populations as to how frequently genetic testing is performed for these drugs and the benefit this will provide in undertaking a precision medicine approach in this population.

A22: Knock out of ABCC5 gene using the CRISPR-CAS9 system increases gemcitabine sensitivity in pancreatic cancer (PANC-1) cells

Piyush Bugde[1], Timothy Lawrence[1] Fabrice Merien[1, 2], Jun Lu[1, 3] , Dong-Xu Liu[1] and Yan Li[1, 3]
[1]School of Science, Auckland University of Technology, Auckland, NZ, [2]AUT-Roche Diagnostics Laboratory, Auckland, NZ, [3]School of Interprofessional Health Studies, Auckland University of Technology, Auckland, NZ

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive solid tumours. Gemcitabine (GEM) is used as a first-line treatment for patients with advanced pancreatic cancer. Previously we have shown transient knock down of ABCC5 (encoding MRP5) increases gemcitabine sensitivity in pancreatic cancer cells. The primary aim of this study is to determine whether knocking out ABCC5 gene by the CRISPR (clustered regularly interspaced short palindromic repeats)-CAS9 (CRISPR associated protein 9) system could increase sensitivity to gemcitabine in human pancreatic cancer PANC-1 cells. The PANC-1 cells were transfected with CAS9 protein/ABCC5 guide-RNA ribonucleoprotein complexes through liposome-mediated delivery. From the mixed population of cells, single cell knock out clones were selected using the limiting dilution method. The efficiency of ABCC5 gene disruption was then assessed by flow cytometry analysis of cell surface MRP5 immunostaining. The accumulation of a specific ABCC5 substrate 2',7'-Bis-(2-Carboxyethyl)-5-(and-6)-Carboxyfluorescein (BCECF) was determined in the single cell knock out clones and wild type PANC-1 cells. MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was undertaken to determine gemcitabine sensitivity, and half-inhibitory concentration (IC_{50}) of gemcitabine was calculated. Knocking out ABCC5 significantly decreased MRP5 surface staining by 72.2± 1.07% ($P < 0.05$). For functional studies of ABCC5, cellular accumulation of BCECF in MRP5-knock out clones increased by 70.24 % ± 1.49 % ($p<0.01$) compared with those in wild type. The IC_{50} values of gemcitabine against PANC-1 cells were significantly lower in MRP5 knock-out cells than those in wild type. These results further confirm that MRP5 confers resistance to gemcitabine and knocking out ABCC5 gene significantly increases the sensitivity of PANC1 cells to gemcitabine.

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2. Bugde, P., et al., *The therapeutic potential of targeting ABC transporters to combat multi-drug resistance.* Expert Opinion on Therapeutic Targets, 2017. **21**(5): p. 511-530.
3. Long, J., et al., *Overcoming drug resistance in pancreatic cancer.* Expert Opin Ther Targets, 2011. **15**(7): p. 817-28.
4. Hagmann, W., et al., *Membrane drug transporters and chemoresistance in human pancreatic carcinoma.* Cancers (Basel), 2010. **3**(1): p. 106-25.

Notes

A23: Metformin in Combination with Crizotinib as an Anti-Cancer Treatment for EML4-ALK+ Non-Small Cell Lung Cancer.

Abigail R Bland[1], John C Ashton[1]. Department of Pharmacology and Toxicology, University of Otago[1], Dunedin, Otago, New Zealand.

Introduction. Lung cancer causes approximately 1.5 million cancer deaths per year. Oncogenic receptor tyrosine kinases have been discovered to play a role in the onset and progression of cancer, and a mutated form of one such receptor is involved in 2-7% of lung cancer; the EML4-ALK chromosomal rearrangement. Crizotinib is an effective inhibitor of EML4-ALK, but resistance usually develops around 12 months after beginning treatment. Novel strategies to overcome this are being explored and one such treatment is the hypoglycemic agent, metformin. Epidemiological studies show that metformin reduces the risk of cancer and recent pre-clinical work has been undertaken to explore this mechanism.
Aim. This work aimed to determine the cytotoxicity and mechanism of a crizotinib/metformin combination in EML4-ALK+ lung cancer.
Methods. The cytotoxicity of crizotinib, metformin and the combination was examined using EML4-ALK+ cells (H3122), ALK-/KRAF+ cells (A549) and crizotinib-resistant (CR-H3122) cells using the sulforhodamine B assay. IGF-1 (100 ng/mL) was added to the cytotoxicity assays to examine if metformin interacts with IGF-1 signaling. Western blots examined changes in ALK, p-ALK, mTOR and p-mTOR.
Results. ALK+ cells were more sensitive to crizotinib compared to ALK- cells (IC_{50}'s of 0.16 µM vs. 1 µM). The addition of metformin (5 mM) reduced cell viability by ˜30% at lower doses of crizotinib. This effect was also observed in resistant cells. IGF-1 produced a 20% increase above baseline at lower crizotinib doses and metformin partially rescues this effect. Mechanistically it appears metformin does not inhibit p-ALK instead decreases p-mTOR.
Conclusion. At low doses of crizotinib, metformin adds to the reduction in cell viability, however this effect is lost at higher doses of crizotinib. It appears metformin does not act directly on ALK, however, produces its effect downstream. This provides rationale to advance towards more molecular mechanistic studies and *in vivo* lung cancer models.

A24: Evaluation of a study design to test the Intact Nephron Hypothesis

Sudeep Pradhan, Daniel F.B. Wright, Stephen B. Duffull. School of Pharmacy, University of Otago[1], Dunedin, Otago, New Zealand.

Introduction. Renal dose adjustment generally assumes a linear relationship between renal drug clearance and glomerular filtration rate (GFR). The theory underpinning this practice is the Intact Nephron Hypothesis (INH) [1]. A recent review by our group [2] suggested that the INH may not be a suitable general model for renal drug clearance.
Aims. The aim of this study was to evaluate designs to test the INH.
Methods. We used the protocol recommended by the European Medicines Agency (EMA) for renal drug studies as the study design for this work [3]. The design compares the clearance of a drug to the clearance of a GFR probe (measured isotopic GFR). Two renal clearance models were proposed to describe the relationship between drug CL and probe CL, (M1) a nonlinear model [4] and (M2) a linear model based on the INH [1]. We conducted a series of simulations to assess the performance of the EMA study design in terms of the ability to identify a departure from the INH. The number of subjects for each simulated study were n = 24, 120, 240, 480, 1080. The studies were replicated 1000 times. The relative standard error (RSE), power and bias were used to assess the EMA study design.
Results. The estimated model parameters for M1 and M2 consistently had RSE less than 32% and were unbiased for all scenarios tested. The EMA study design had a power > 80% to discriminate between M1 and M2 under all scenarios.
Discussion. The EMA study design performed well for power and provided unbiased and precise parameter estimates. We note, however, that the circumstances of the test were in the ideal setting where mGFR (via isotopic determination) was available. The performance of the EMA study design needs to be re-evaluated for circumstances when eGFR is used instead of mGFR.

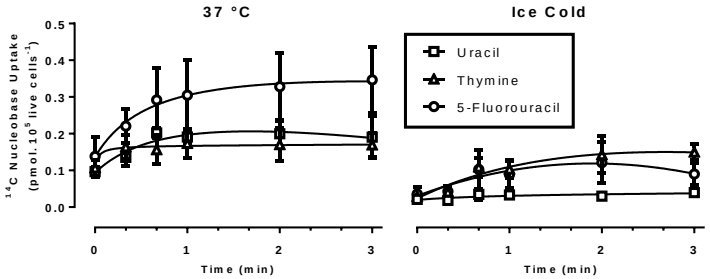
1. Bricker N S et al (1960) Am J Med 28:77-98
2. Pradhan S et al (2018) Clin Pharmacol Ther [Under review]
2. European Medicines Agency (2014) Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/02/WC500200841.pdf
3. Wright D F B et al (2017) Br J Clin Pharmacol 83(9):1869-72

Notes

A25: Characterization of Nucleobase Transport in Human Cells

Situmorang M[1], Helsby N [1,2], Burns K[1]. Department of Molecular Medicine and Pathology, University Of Auckland[1], Auckland, New Zealand. Auckland Cancer Society Research Centre[2], Auckland, New Zealand

Introduction. 5-Fluorouracil (5-FU), an analogue of the endogenous pyrimidine nucleobases uracil and thymine, is used widely for the treatment of gastrointestinal and breast cancers. Little is known about the mechanisms that underpin transport of these nucleobases in human cells.



Aims. To investigate the transport of nucleobases (uracil, thymine, 5-FU) in cells derived from human carcinomas.

Methods. The transport of 5 µM [^{14}C] Uracil, [^{14}C] Thymine, and [^{14}C] 5-FU into a human intestinal carcinoma cell line (HCT 116) was investigated over time using *in vitro* cell culture. Incubations were undertaken at both 37 °C and on ice.

Results. Uptake of both 5-FU and uracil at 37° C was saturable and showed first order kinetics at time points <1 minute. In contrast, uptake of thymine was saturated at 20 seconds at 37 °C. The maximum uptake of 5-FU into HCT 116 cells (0.37 pmol.10^5 live cells^{-1}, 95% CI 0.28-0.49 pmol.10^5 live cells^{-1}) was higher than that of thymine (0.17 pmol.10^5 live cells^{-1}, 95% CI 0.14-0.22 pmol.10^5 live cells^{-1}) and uracil (0.22 pmol.10^5 live cells^{-1}, 95% CI 0.17-0.27 pmol.10^5 live cells^{-1}). When the incubations were repeated on ice uptake of 5-FU and uracil was substantially decreased and remained saturable only for 5-FU, with the data for uracil appearing linear. Interestingly, the maximum uptake of thymine on ice (0.19 pmol.10^5 live cells^{-1}, 95% CI 0.15-0.27 pmol.10^5 live cells^{-1}) was comparable to that observed at 37 °C, but saturated more slowly at the lower temperature.

Discussion. Uptake of the nucleobases uracil, thymine, and 5-FU is consistent with transporter mediated-processes in HCT 116 cells. However differences in the kinetics of uptake for each nucleobase are apparent, and may be due to divergent transporter affinities.

A26: Development of predictive model for lean liver volume, a potential scaler for in *vitro-in vivo* extrapolation of drug clearance

Jaydeep Sinha[1], Stephen Duffull[1], Bruce Green[2], T. K. Ponnuswamy[3], B. Devanand[3], M. Ramanathan[4], S. Ramalingam[3], Hesham Al-Sallami[1]. Otago Pharmacometrics Group, University of Otago [1], Dunedin, New Zealand; Model Answers R&D Pty Ltd [2], Brisbane, Australia; PSG Institute of Medical Sciences and Research [3], Coimbatore, India; PSG College of Pharmacy [4], Coimbatore, India

Introduction. *In vitro-in vivo* extrapolation (IVIVE) is a useful technique to predict drug clearance (*CL*) in patient populations that are not routinely studied during drug development (e.g. obese patients). Standard IVIVE approach for hepatically-cleared drugs considers total liver volume (*LV*) to scale *in vivo CL* from *in vitro* data. However, *LV* may not always represent the metabolically active volume of the liver, particularly in patients with steatosis (liver fat > 5% of *LV*). Instead, lean liver volume (*LLV*) may be a more accurate scaler especially in obese patients who often have a degree of steatosis.

Aims. To develop and evaluate a predictive model of LLV from measurable patient characteristics.

Methods. LLV data (single measurement) were derived by subtracting the liver fat from *LV*, measured by computed tomography in 100 adult Indian participants and modeled using NONMEM v7.3. Available covariates were weight (*WT*), fat-free mass (*FFM*), *SEX*, clinical chemistry, liver and kidney function markers. Model selection and evaluation were done by standard statistical diagnostics and plots, e.g. log-likelihood ratio test, visual predictive checks, and biological relevance.

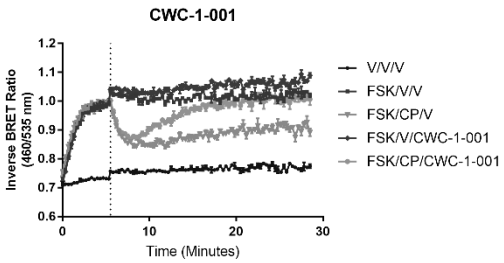
Results. FFM and SEX were retained in the final model. The final model indicates that individual LLV changes proportionally with the change in FFM from a population standard LLV of 1440 (males) and 1280 (females) mL.

Discussion. A model to predict LLV from SEX and FFM was developed and evaluated. The model could be used for IVIVE of drug *CL* particularly in obese patients with steatosis after appropriate evaluation.

Notes

A27: Evaluation of Novel Allosteric Modulators of the Type-1 Cannabinoid Receptor.

Jamie J. Manning[1], Savanah L. Saldana[2], Christopher W. Cunningham[2], Michelle Glass[1]. Dept. Pharmacology, Otago University[1], Dunedin, New Zealand; Dept. Pharmaceutical Sciences, Concordia University[2], Mequon, WI, USA



Introduction. The CB_1 cannabinoid receptor has an allosteric site with which allosteric modulators such as ORG27569 can interact, to alter orthosteric drug responses[1].
Aims. To perform a systematic characterisation of a series of 8 novel compounds based on the structure of ORG27569. This project characterised their effects on orthosteric binding, cAMP signalling, and β-arrestin 1 and 2 translocation alone or upon co-stimulation with orthosteric ligands.
Methods. Using radioligand binding assays we determined the influence of the series on binding of [^{3}H]-CP-55,940. Real-time Bioluminescent-resonance Energy Transfer assays (BRET) assays were used to report changes in cAMP production and β-arrestin-1/2 translocation to the membrane.
Results. Most compounds facilitated increases in orthosteric ligand binding. A unique temporal desensitisation of agonist-mediated cAMP signalling was observed for some compounds, while others were inactive. Finally, compounds that were inactive in modulating cAMP signalling may exhibit 'biased' inhibition of β-arrestin-1 and 2 activity.
Discussion. These data indicate that these compounds exhibit positive allosteric modulation of ligand binding, but allosteric inhibition of cAMP production and β-arrestin-1/2 recruitment. Intriguingly, some compounds appear to simultaneously display allosteric inhibition of agonist-induced β-arrestin recruitment but inactivity in the cAMP pathway. Others show an interesting allosteric inhibition of cAMP signalling which returns the receptor to baseline activity. These effects differ from ORG27569 in a manner that suggests pronounced differences in signalling kinetics.

Cawston, E. E., Redmond, W. J., Breen, C. M., Grimsey, N. L., Connor, M., & Glass, M. (2013). Real-time characterization of cannabinoid receptor 1 (CB 1) allosteric modulators reveals novel mechanism of action. British journal of pharmacology, 170(4), 893-907.

A28: Antihelminthic benzimidazoles mebendazole and albendazole kill cancer cells via classical apoptosis

Jakeb SSM Petersen, Sarah K Baird. Department of Pharmacology & Toxicology, University of Otago, Dunedin, N.Z.

Introduction. Antihelminthic benzimidazole drugs mebendazole and albendazole are commonly used to treat a variety of worm infestations in humans. Their mechanism of action against helminths is well-established and involves the inhibition of microtubule formation. Mebendazole has recently shown promising results in pre-clinical *in vitro* and *in vivo* cancer studies and is currently in Phase I trials for treatment of glioma. However, the way in which it causes cell death in cancer cells has not been fully explored.
Aims. To investigate the *in vitro* anticancer mechanism of action of mebendazole and a structural analogue albendazole. Methods. The two drugs were screened for cytotoxicity in three cancer cell lines (MDA-MB-231, MCF-7, HT-29) and one non-transformed mesenchymal stem-cell line (RCB2157), using the MTT assay. Their effects on the cell cycle and cell death mechanisms were further analysed in HT-29 cells using flow cytometry and fluorescent microscopy and spectroscopy.
Results/Discussion. Mebendazole and albendazole were found to selectively kill cancer cells, being most potent in the colorectal cancer cell line HT-29, with IC_{50} values of 1.3 ± 0.1 µM and 1.4 ± 0.1 µM, respectively. Both mebendazole and albendazole induced caspase-3 activation 3-4-fold. Phosphatidylserine exposure, mitochondrial and lysosomal membrane permeability and ROS production were all significantly increased compared to control and peaked at 24 hours, with DNA fragmentation increasing in a time-dependent manner peaking at 48 hours. Using Hoechst 33342 staining, nuclear features of apoptosis such as chromatin condensation were found following treatment with both drugs. Cell cycle arrest in the G2/M phase was found, and tubulin structures were significantly altered. Mebendazole and albendazole appear to cause cancer cell death via a mechanism of classical apoptosis and cell cycle arrest, which may originate from the destabilisation of microtubules.

Notes

A29: Attenuation of ischaemia reperfusion injury in hypertrophic hearts with a novel carbon monoxide releasing molecule, oCOm-21.

SH Thwaite, AH Chau, JC Harrison, MI Read, IA Sammut. Department of Pharmacology and Toxicology, University of Otago, Dunedin, New Zealand.

Introduction. Acute cardiovascular interventions are common major surgical procedures used worldwide. However, these procedures can cause cardiac damage, specifically ischaemia-reperfusion (IR) injury. Additionally, hypertrophic cardiomyopathy potentiates these perioperative complications.
Aim. Previous work by our lab group established controlled release of low-dose carbon monoxide (CO) from the novel, organic CO releasing molecule, oCOm-21, attenuates IR injury in isolated healthy rat hearts. The present study was conducted to determine the cardioprotective potential of oCOm-21 as a conditioning agent in hypertrophic hearts.
Methods. Male CYP1A1-Ren2 rats (n=30, 16-weeks/300-380 g) were fed an indole-3-carbinol (0.167 %) diet for eight-weeks to induce hypertension and cardiac hypertrophy. Cardiac hypertrophy was confirmed by heart weight to body weight ratio standardisation at termination. Hearts were perfused retrograde through the aorta in Langendorff mode with Krebs-Henseleit buffer to allow for haemodynamic assessment and randomly allocated into treatment groups; vehicle control, oCOm-21 (1 & 3 µM) and DB-21 (10 µM) (non-CO releasing analogue of oCOm-21). Hearts were dosed for ten-minutes prior to a 30-minute warm global ischaemic episode, followed by a 60-minute reperfusion period. Cardiac function was determined by the placement of an intraventricular balloon. The degree of myocardial injury was assessed through the level of lactate dehydrogenase (LDH) leakage in the coronary effluent with a LDH activity assay.
Results. Pre-treatment with oCOm-21, compared to vehicle and DB-21 controls at 60-minutes significantly ($p < 0.05$) improved the haemodynamic parameters of left ventricular developed pressure (60 – 75% increase), systolic pressure (80 – 100 % increase 1 µM & 20 – 30 % increase 3 µM) and end-diastolic pressure (40 – 60% decrease 3 µM). Further, oCOm-21 pre-treatment significantly ($p < 0.05$) decreased myocardial cellular injury upon reperfusion seen by the prevention of increased LDH leakage upon reperfusion compared to controls.
Discussion. In conclusion, this study provides critical evidence for further development and testing of oCOm-21 as a pre-conditioning agent for use in acute cardiovascular interventions to attenuate perioperative complications associated with IR injury.

A30: Vancomycin dosing, target attainment, and optimisation in patients receiving high-flux haemodialysis

Carl M Kirkpatrick (1) Katrina Hui (1), Kashyap Patel (2), David CM Kong (1), and KAOS group (3,4)
1. Centre for Medicine Use and Safety, Monash University, Melbourne; 2. Certara L.P, New Jersey; 3. Royal Melbourne Hospital, Melbourne; 4. Western Hospital, Melbourne

Introduction: Vancomycin is the most commonly prescribed intravenous (IV) antibiotic in the high-flux haemodialysis (HFHD) setting.
Aims: To i) evaluate fixed dosing (FD) and weight-based (i.e. mg/kg) dosing (WBD) of vancomycin in the HFHD setting, and ii) develop a population pharmacokinetic (PK) model for vancomycin in HFHD to examine the probability of target attainment (PTA) of several dosing regimens of vancomycin to optimise dosing.
Methods: Data were collected retrospectively from 73 patients receiving HFHD from two Victorian hospitals across a 5 year period who received vancomycin via FD or WBD regimens. Pre-haemodialysis vancomycin concentrations were evaluated. A population PK model was developed using non-linear mixed effects modelling. Clinically relevant dosing regimens were evaluated using Monte Carlo simulations to determine the PTA for vancomycin over 8 days. The PTA for vancomycin was an AUC/MIC_{24hour} ≥400 for micro-organisms with an MIC of 1mg/L.
Results: Compared to WBD group, the FD group had a greater proportion of pre-haemodialysis concentrations <15 mg/L, but a smaller proportion of pre-haemodialysis concentrations >25 mg/L. A two-compartment model with a proportional error model and between-subject variability on non-HFHD clearance (CL), central compartment volume (V1) and peripheral compartment volume (V2). Lean body weight was included as a covariate on non-HFHD CL, V1 and V2. FD vancomycin resulted in the PTA being ≤90% for most days of therapy, while WBD resulted in the PTA being ≥90% on all days. The optimized schedule was a WB loading dose of 30mg/kg and a FD maintenance dose of 1g achieved a PTA ≥90% on all days when a HFHD session.
Discussion: In the HFHD setting, a WB loading dose of 30mg/kg and a maintenance dose of 1g on HFHD days appears optimal. A delay of greater than 1 day between LD and first HFHD session requires an additional 500mg dose of vancomycin on each non-HFHD day to ensure the PTA remains above 90%

Notes

A31: Anticancer efficacy of evofosfamide in head and neck cancer tumour models

Francis W Hunter[1,2], Tet-Woo Lee[1,2], Avik Shome[1], Tao Wang[1], Way W Wong[1], Maria K Kondratyev[3], Arthur Liu[4], Brad Wouters[3], Michael A Curran[4], William R Wilson[1,2], Stephen MF Jamieson[1,2,5]. Auckland Cancer Society Research Centre, University of Auckland[1], Auckland, New Zealand; Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland[2], Auckland, New Zealand; Princess Margaret Cancer Centre[3], University Health Network, Toronto, ON, Canada; Department of Immunology, University of Texas[4], MD Anderson Cancer Center, Houston, TX, United States; Department of Pharmacology and Clinical Pharmacology, University of Auckland[5], Auckland, New Zealand.

Introduction. Evofosfamide (TH-302) is a clinical-stage hypoxia-activated prodrug, designed to selectively target a potent DNA crosslinking nitrogen mustard to the poorly oxygenated regions of tumours and spare normal tissue. Evofosfamide has been tested in 27 clinical trials to date, but despite strong evidence that hypoxia is a marker of poor prognosis and failure of chemoradiotherapy in head and neck squamous cell carcinoma (HNSCC), it has not previously been evaluated either clinically or preclinically in HNSCC.
Aims. To evaluate the anticancer activity of evofosfamide in HNSCC cell lines and tumour models and perform an initial investigation of biomarkers that can predict sensitivity to evofosfamide in HNSCC
Methods. The antiproliferative activity of evofosfamide was investigated in 22 HNSCC cell lines genomically characterised by RNA sequencing. The anticancer efficacy of evofosfamide was assessed in cell line xenograft, patient-derived xenograft and syngeneic tumour models of HNSCC as a single agent and combined with radiotherapy or anti-CTLA4 therapy. Tumour hypoxia status was determined by pimonidazole staining.
Results. Evofosfamide potently inhibited cell proliferation in HNSCC cells selectively under hypoxia with activity being associated with expression of a proliferation metagene. Antitumour efficacy was observed with single agent evofosfamide in patient-derived xenograft models and in combination with radiotherapy in cell line xenograft and patient-derived xenograft models. Evofosfamide also delayed tumour growth when combined with anti-CTLA4 in a syngeneic tumour model and reduced hypoxia in nodal metastases in an orthotopic HNSCC cell line tumour model.
Discussion. These data suggest that evofosfamide is active against HNSCC tumours, although there is a need to clearly define appropriate biomarkers to identify the tumours that will be most sensitive to evofosfamide therapy and support the clinical evaluation of evofosfamide in HNSCC patients.

A32: Prediction of patients with severe to life threatening fluoropyrimidine-induced gastrointestinal toxicity using the thymine challenge test

Nuala Helsby[1], Kathryn Burns[1], Claire Bonnet[1], Soo Hee Jeong[1], Katrina Sharples[2], John Duley[3], David Porter[4], Mike Findlay[4]. Molecular Medicine and Pathology, University of Auckland[1], NZ, Statistics, University of Otago[2], NZ, School of Pharmacy, University of Queensland[3], Australia, Auckland City Hospital, Auckland[4], NZ

Introduction. Most patients with severe fluoropyrimidine toxicity do not have inherited DPD deficiency and phenotypic tests may be more helpful than genotyping. We have recently reported that a thymine challenge detects aberrant pyrimidine pharmacokinetics in a majority of toxicity cases compared with healthy volunteers [Duley et al 2018], but it is not known if this test can discriminate patients who tolerate treatment (non-cases) from toxicity cases.
Aims. A case-control study (ACTRN12615000586516) was undertaken to assess the ability of the thymine test to discriminate cases of severe toxicity from patients who tolerated standard treatment with 5-FU or capecitabine.
Methods. Patients (n= 37) received a 250 mg (PO) dose of thymine and a cumulative urine sample collected for 0-4 h. The amount of thymine (THY) and metabolite dihydrothymine (DHT) in the urine were determined by LC/MS. Genomic DNA was analysed for *DPYD* gene variants. Cases (n= 9) and non-cases (n= 23) of severe gastrointestinal toxicity were defined based on Common Terminology Criteria for Adverse Events v4.0. Logistic regression was used to obtain a predicted probability of being a case and ROC curves were used to compare continuous values of the test.
Results. THY/DHT ratios were elevated in cases but were not significantly different (P = 0.07) between cases and non-cases. Creatinine clearance (CrCl) was significantly lower (P= 0.0036) in cases, but could not discriminate cases from non-cases adequately. A logistic regression model gave estimated ORs of 1.3 [95%CI 0.8-2.1] and 0.92 [95%CI 0.85-0.99] for THY/DHT and CrCl respectively. We used the predicted probability of toxicity from the logistic regression to construct an ROC curve for discrimination of cases from non-cases; the area under the ROC curve was 0.88 (95%CI 0.73-1.0). For a cutoff giving a specificity of 100% the sensitivity of the regression test was 55%.
Discussion. Thymine challenge test may be useful as a potential phenotypic test to detect risk of excessive fluoropyrimidine toxicity, since individuals with high THY/DHT ratio combined with relatively poor renal function appear to have a higher chance of fluoropyrimidine-induced GI toxicity.
Duley et al (2018) Ther Drug Monit. May 24. doi: 10.1097/FTD.0000000000000532. [Epub ahead of print].

Notes

A33: Fits or falls – it's all in the genes/jeans

Phenytoin toxicity – using first principals to elicit the cause

Chris Cameron, Capital & Coast DHB, University of Otago Wellington

When faced with cases of Type A adverse drug reactions, due to supra-therapeutic drug concentrations, eliciting the cause can be straightforward or less so. By returning to first principals of the limited number of causes of an unexpected supra-therapeutic drug concentration the health professional should always be able to elicit the cause, even if there seems to be no obvious case, or a "more obvious" cause.

In this presentation, there are two case presentations with a discussion about the importance of returning to the first principals of clinical pharmacokinetics to elicit the cause of phenytoin toxicity and suggestions for management.

A34: Do concomitant medicines impede safe and effective personalised lung cancer treatment in NZ?

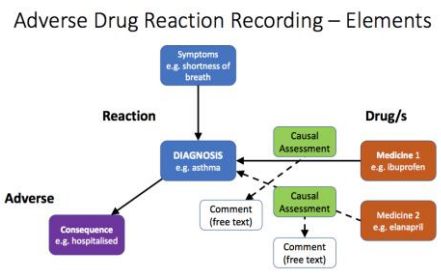
Mark J McKeage, Pharmacology and Clinical Pharmacology and ACSRC, University of Auckland, Auckland, NZ.

Lung cancer is the leading cause of cancer death and the fifth most commonly registered cancer in NZ. Between 2010 and 2014, PHARMAC funded the first genotype-directed personalised lung cancer treatments in NZ, the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib, and the Ministry of Health published national guidelines for EGFR mutation testing to identify patients for these treatments. To gain understanding of genetically defined subtypes of non-small cell lung cancer (NSCLC) in NZ, and determine the local uptake and impact of these genotype-directed personalised treatments, we undertook an observational, retrospective, data-linkage study of electronic healthcare administrative database extractions. EGFR mutations were found in 22% of NSCLC patients, equating to a national incidence of EGFR mutation-positive NSCLC in NZ of approximately 300 new cases per year. NZ patients with EGFR mutation-positive NSCLC were often females (70% of cases) and never smokers (44% of cases). Introduction of EGFR mutation testing and EGFR-TKIs has begun to reduce mortality in NZ, but patient outcomes are poorer than expected. Median overall survival of NZ patients treated with erlotinib or gefitinib for EGFR mutation-positive NSCLC was about 18 months, which was lower than achieved in international randomised controlled trials (up to 30.9 months). Up to 25% of NZ patients prematurely discontinued erlotinib and gefitinib within three months of starting treatment for reasons that are poorly understood. These findings suggest that health gains from personalised lung cancer treatment so far appear more limited than expected in NZ. Erlotinib and gefitinib display pH-dependent solubility and oral absorption, extensive metabolism by cytochrome P450 3A4 (CYP3A4) and marked pharmacokinetic interaction on co-administration with medicines that induce or inhibit gastric acid acidity or CYP3A4. These findings led us to hypothesise that the concomitant use of medicines that can interact with erlotinib or gefitinib is prevalent in NZ lung cancer patients, and contributes to their poor survival outcomes and serious adverse events. This hypothesis will be tested in a proposed observational, data-linkage, retrospective, national cohort study of all patients prescribed erlotinib or gefitinib for the treatment of EGFR mutation-positive NSCLC in NZ over a 10-year period, estimated at about 2000 patients, that now awaits funding.

Notes

A35: Recording Adverse Drug Reactions in New Zealand.

Matthew Doogue[1,2], Paul Chin[1,2], Colin Simpson[3], Gareth Frew[4], Ben Hudson[5], Claire Heppenstall[1]. Departments of Medicine[1] and General Practice[5] University Of Otago, Christchurch; Department of Clinical Pharmacology[2] and Canterbury Community Pharmacy Group[4], Canterbury District Heath Board[2], Christchurch; Faculty of Health, Victoria University of Wellington[3], Wellington, New Zealand.



Introduction. Adverse drug reactions (ADRs) are diagnoses inconsistently recorded by clinicians many times across many systems. This leads to errors that harm patients and cost the health system. Underpinning this is a poor record keeping system. Two important changes are happening, firstly New Zealand is adopting standards to record ADRs and secondly providers are adopting electronic prescribing systems capable of providing ADR alerts at the point of prescribing.

Aims. To design an ADR recording process to implement draft NZ ADR standards within existing workflows.

Method. Existing processes, workflows and systems in hospital and community were evaluated. Limitations in data quality and utility of current systems were mapped to clinical requirements.

Results. The data elements to record ADRs are codable and could be captured with minor modifications to existing systems. Common standards are required to share the data between systems. New ADRs should be recorded comprehensively at the time they happen. The processes used for medicines reconciliation are suitable for recording historical ADRs.

Discussion. With common standards valid and reliable ADR records could be created and shared between systems. The medication reconciliation process is an opportunity to reconcile historical ADRs if it is well supported. If effective this is scalable and would consequently improve patient outcomes and reduce costs. The potential gains are shown by the ADR burden with 10% of hospital admissions being because of ADRs, half of which are preventable.

Notes