

Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists and Carney Symposium

New Zealand Annual Scientific Meeting



Camelot Room, The Chateau on the Park
189 Deans Avenue, Riccarton, Christchurch

28 – 30 August 2011



SCIENTIFIC AND SOCIAL PROGRAMME

SUNDAY, 28 AUGUST 2011

6.00 – 7.30 pm *REGISTRATION AND OPENING RECEPTION - CAMELOT ROOM,
THE CHATEAU ON THE PARK*

MONDAY, 29 AUGUST 2011

8:00 - 9:00 am *REGISTRATION*

9:00 am Welcome and Announcements

1. ASCEPT PLENARY/SESSION 1

CHAIR: EVAN BEGG

9:05 am 1.1 **PLENARY SPEAKER**

Carl Kirkpatrick, Pharmacy and Pharmaceutical Sciences, Monash University, Melbourne, Australia

Taking the piss in the critically ill: when 1+1 ≠ 0.5

10:00 am 1.2 *Paul Chin, Christchurch Hospital, Christchurch*

The performance of eGFR formulae for estimating gentamicin clearance

10.20 am 1.3 *Lisa Stamp, University of Otago, Christchurch*

Starting dose, but not maximum maintenance dose, is a risk factor for allopurinol hypersensitivity syndrome: a proposed nomogram for safe starting dose of allopurinol

10.40 am *Morning Tea*

2. ASCEPT SESSION 2

CHAIR: JANE VELLA-BRINCAT

- 11:15 am 2.1 *Dan Wright, University of Otago, Dunedin*
A population pharmacokinetic model for allopurinol in gout patients
- 11:30 am 2.2* *Julia Korell, University of Otago, Dunedin*
Development and application of a pharmacokinetic model for the glycation of albumin
- 11:45 am 2.3 *Hesham Al-Sallami, University of Otago, Dunedin*
Estimating fat free mass in children
- 12:00 noon 2.4* *Claire Johnston, Royal North Shore Hospital, NSW, Australia*
Population pharmacokinetics of gentamicin in older people: The impact of frailty
- 12:15 pm 2.5 *Evan Begg, University of Otago, Christchurch*
Extended-interval gentamicin dosing in adult patients with impaired renal function – should the dose interval be extended further?
- 12:30 pm *Lunch*

3. ASCEPT SESSION 3

CHAIR: BERIT JENSEN

- 1:30 pm 3.1 *Nick Holford, University of Auckland, Auckland*
Demonstration of symptomatic and disease modifying effects of levodopa in Parkinson's disease using the ELLDOPA study
- 1:45 pm 3.2 *Mark McKeage, University of Auckland, Auckland*
Mass balance, metabolism and excretion of [14C]ASA404 in cancer patients
- 2:00 pm 3.3 *Andrew McKean, Hillmorton Hospital, Christchurch*
Is it NICE to monitor lithium routinely?
- 2:15 pm 3.4 *Ganeesan Kichenadasse, Flinders Medical Centre, Adelaide, South Australia*
In silico assessment of erlotinib as a potential inhibitor of CYP3A-mediated drug clearance
- 2:30 pm 3.5 *Paul Zufferey, University of Otago, Dunedin*
Fondaparinux: not so simple renal elimination
- 2:45 pm *Afternoon Tea*

* For consideration for student prize

4. ASCEPT SESSION 4

CHAIR: PAUL CHIN

- 3.15 pm 4.1 *Steve Duffull, University of Otago, Dunedin*
The current funding model leaves pharmacists caught in the struggle between justice and non-maleficence
- 3:30 pm 4.2* *Ernieda Hatah, University of Otago, Dunedin*
General practitioners' perceptions of pharmacist prescribing in New Zealand
- 3.45 pm 4.3 *Jane Vella-Brincat, Christchurch Hospital, Christchurch*
The pharmacokinetics of the antiemetic cyclizine, and how it feels to be subjected to blood sample orientated research, in palliative care
- 4:00 pm 4.4 *Melanie Gamble, Christchurch Hospital, Christchurch*
An audit of the antimicrobial guideline for the treatment of acute epiglottitis in adults
- 4.15 pm 4.5 *Pamela Buffery, Christchurch Hospital, Christchurch*
A retrospective pharmacokinetic review of the busulphan monitoring service at Christchurch Hospital

4:30 pm

ASCEPT AGM

7.00 pm

CONFERENCE DINNER IN THE CAMELOT ROOM, THE CHATEAU ON THE PARK

* For consideration for student prize

TUESDAY, 30 AUGUST 2011



ASCEPT/7TH ANNUAL CARNEY PHARMACOGENOMICS SYMPOSIUM

8:30 – 9:00 am **CARNEY REGISTRATION**

9:00 am Welcome and Announcements

5. ASCEPT SESSION 5

CHAIR: CARL KIRKPATRICK

- 9.05 am 5.1* *Christine Dixon, The University of Queensland, Queensland, Australia*
A high-throughput screen for novel drugs acting on the $\alpha 2\beta 2\gamma 1$ GABA_A receptor
- 9.20 am 5.2* *Matthew Bull, University of Auckland, Auckland*
Development of perforin inhibitors as pharmacological agents
- 9.35 am 5.3* *Nancy Jong, University of Auckland, Auckland*
Oxaliplatin transport mediated by organic cation/carnitine transporters OCTN1 and OCTN2 in over-expressing HEK293 cells and rat dorsal root ganglion neurons
- 9.50 am 5.4* *Feifei Feng, University of Otago, Dunedin*
Ketotifen uptake by rat brain endothelial (RBE4) cells is stereoselective
- 10.05 am 5.5* *Sarah Bushby, University of Otago, Dunedin*
Forensic investigations of a psychotropic drug using maggots in a brain model
- 10.20 am 5.6* *Chakradhar Lagishetty, University of Otago, Dunedin*
Biomarkers of aging to predict drug clearance in the elderly - a pilot study
- 10:35 am *Morning Tea*

* For consideration for student prize

6. CARNEY PLENARY/SESSION 1

CHAIR: MARTIN KENNEDY

- 11.05 am 6.1 *Elizabeth Phillips, Institute for Immunology and Infectious Diseases, Murdoch University, Perth, Australia*
Translating pharmacogenetics into the clinic
- 12.00 pm 6.2 *Richard Gearry, University of Otago, Christchurch*
GWAS, G WHIZZ - an ignorant approach to genetics?
- 12.20 pm 6.3 *Berit Jensen, University of Otago, Christchurch*
LC-MS in clinical pharmacology and toxicology – from niche projects to routine screening
- 12.40 pm **PRESENTATION OF FRED FASTIER TRUST STUDENT PRIZE**
- 12.45 pm *Lunch*

7. CARNEY SESSION 2

CHAIR: REBECCA ROBERTS

- 1:45 pm 7.1 *Hannah Kennedy, Christchurch Hospital, Christchurch*
Identification of multiple sclerosis patients who are non-responsive to therapeutic IFN β : an assay to allow more informed treatment options.
- 2.00 pm 7.2 *Mei Zhang, University of Otago, Christchurch*
Determination of 6-thioguanine nucleotides and 6-methylmercaptopurine nucleotides in human red blood cells by LC-MS/MS
- 2.15 pm 7.3 *Nuala Helsby, University of Auckland, Auckland*
CYP2C19 poor metabolisers: who and why?
- 2.30 pm 7.4 *Murray Barclay, University of Otago, Christchurch*
High TMPT enzyme activity does not explain drug resistance due to preferential 6-methylmercaptopurine production in patients on thiopurine treatment
- 2.45 pm *Afternoon Tea*

8. CARNEY SESSION 3

CHAIR: MURRAY BARCLAY

- 3.15 pm 8.1 *Janet Coller, University of Adelaide, Adelaide, Australia*
Association between immune genetic markers and alcohol dependence
- 3.35 pm 8.2 *Rebecca Roberts, University of Otago, Dunedin*
The benzbromarone story - is CYP2C9 genotype relevant to prescribing?
- 3.55 pm 8.3 *Patrick Gladding, University of Auckland, Auckland*
Personalised thienopyridine therapy: the cost effectiveness of genetic testing for CYP2C19 variants to guide treatment in patients with acute coronary syndromes
- 4.15 pm **CLOSING STATEMENT AND SESSION CLOSED**

REGISTRANTS 2011

SURNAME	FIRST NAME	DEPARTMENT	INSTITUTE/ORGANISATION
Acharam	Hannah	Pathology	University of Otago, Christchurch
Al-Sallami	Hesham	School of Pharmacy	University of Otago, Dunedin
Assawasuwannakit	Piyanan	School of Pharmacy	University of Otago, Dunedin
Aitchison	Alan	Pathology	University of Otago, Christchurch
Balasubramanain	Diana	Pathology	University of Otago, Christchurch
Barclay	Murray	Clinical Pharmacology	Christchurch Hospital, Christchurch
Begg	Evan	Clinical Pharmacology, Department of Medicine	University of Otago, Christchurch
Borrie	Tracey	Clinical Pharmacology	Christchurch Hospital, Christchurch
Buffery	Pamela	Clinical Pharmacology	Christchurch Hospital, Christchurch
Bull	Mathew	Auckland Cancer Society Research Centre	University of Auckland, Auckland
Bushby	Sarah	National School of Pharmacy	University of Otago, Dunedin
Cameron	Chris	Clinical Pharmacology	Wellington Hospital, Wellington
Chin	Paul	Clinical Pharmacology	Christchurch Hospital, Christchurch
Coberger	Elle	Clinical Pharmacology	Christchurch Hospital, Christchurch
Coller	Janet	Pharmacology	Medical School North University of Adelaide, South Australia
Coulter	Carolyn	School of Pharmacy	University of Otago, Dunedin
Crawshay	Cherry	Pharmacy	Christchurch Hospital, Christchurch
Dalrymple	Judy	Clinical Pharmacology	Christchurch Hospital, Christchurch
Deng	Sarah (Ziaoyan)	Pathology	University of Otago, Christchurch
Dixon	Christine	Queensland Brain Institute	University of Queensland, Queensland, Australia
Doudney	Kit	Pathology	University of Otago, Christchurch
Duffull	Steve	School of Pharmacy	University of Otago, Dunedin
Feng	Feifei	School of Pharmacy	University of Otago, Dunedin
Gamble	Melanie	Clinical Pharmacology	Christchurch Hospital, Christchurch
Gearry	Richard	Department of Medicine	University of Otago, Christchurch
Gladding	Patrick	Theranostics (NZ)	North Shore Hospital, Auckland
Hart	Joanne	Clinical Risk Management	Medsafe, Wellington
Hatah	Ernieda	School of Pharmacy	University of Otago, Dunedin
Helsby	Nuala	Molecular Medicine and Pathology	University of Auckland, Auckland
Henshaw	Kathryn	Clinical Pharmacology	Christchurch Hospital, Christchurch
Holford	Nick	Pharmacology and Clinical Pharmacology	University of Auckland, Auckland
Jensen	Berit	Clinical Pharmacology, Department of Medicine	University of Otago, Christchurch
Jodczyk	Sarah	Pathology	University of Otago, Christchurch
Johnston	Claire	Clinical Pharmacology and Ageing Research	Royal North Shore Hospital, St Leonards, NSW, Australia
Jong	Nancy	Pharmacology and Clinical Pharmacology	University of Auckland, Auckland
Joyce	Peter	School of Medicine	University of Otago, Christchurch
Kennedy	Hannah	Molecular Pathology	Christchurch Hospital, Christchurch
Kennedy	Martin	Pathology	University of Otago, Christchurch
Kichenadasse	Ganeesan	Flinders Medical Centre	Adelaide, South Australia
Kickpatrick	Carl	Pharmacy and Pharmaceutical Sciences	Monash University, Melbourne, Australia
King	Richard	Biochemistry	Canterbury Health Laboratories, Christchurch
Korell	Julia	School of Pharmacy	University of Otago, Dunedin
Lagishetty	Chakradhar	School of Pharmacy	University of Otago, Dunedin
Lea	Andrew	Genetic Test Evaluation Program	Hayes, Inc., Christchurch
McKeage	Mark	School of Medical Science	University of Auckland, Auckland
McKean	Andrew	Pharmacy	Hillorton Hospital, Christchurch

SURNAME	FIRST NAME	DEPARTMENT	INSTITUTE/ORGANISATION
Millar	Alasdair	Medical Department	Southland Hospital, Invercargill
Miller	Allison	Pathology	University of Otago, Christchurch
Morgan	Angharad		University of Auckland, Auckland
Phillips	Elizabeth	Institute for Immunology and Infectious Diseases	Murdoch University, Perth, Australia
Reith	David	School of Medicine	University of Otago, Dunedin
Roberts	Rebecca	Biochemistry	University of Otago, Dunedin
Robson	Richard		Christchurch Clinical Studies Trust Ltd
Savage	Ruth	Preventive and Social Medicine	University of Otago, Dunedin
Smart	Roger		Douglas Pharmaceutical Ltd, Auckland
Stamp	Lisa	Department of Medicine	University of Otago, Christchurch
Stevenson	Philippa	Pharmacy	Christchurch Hospital, Christchurch
Tucker	Ian	School of Pharmacy	University of Otago, Dunedin
Turkistani	Areej	Pharmacology and Clinical Pharmacology	University of Auckland, Auckland
Vella-Brincat	Jane	Clinical Pharmacology	Christchurch Hospital, Christchurch
Winter	Helen	School of Pharmacy	University of Otago, Dunedin
Wright	Dan	School of Pharmacy	University of Otago, Dunedin
Wynne	Chris		Christchurch Clinical Studies Trust Ltd
Zhang	Mei	Clinical Pharmacology, Department of Medicine	University of Otago, Christchurch
Zufferey	Paul	School of Pharmacy	University of Otago, Dunedin

ASCEPT (NZ) AGM 2011

Agenda of the Annual General Meeting to be held in the Camelot Room, The Chateau on the Park
on Monday, 29 August at 4.30pm

1. Apologies
2. Minutes of 2010 AGM in Christchurch and matters arising
3. President's Report – Evan Begg
4. Treasurer's Report – Paul Chin
5. Other Business

MINUTES OF ASCEPT (NZ) ANNUAL GENERAL MEETING

Monday, 30 August 2010 at 4.45pm in Beaven Lecture Theatre, University of Otago, Christchurch

1) Present: Evan Begg, Paul Chin, Berit Jensen, Murray Barclay, Jane Vella-Brincat, matt Doogue, Janet Coller, Chris Cameron, Paul Fawcett, Simran Maggo, Dan Wright, Mei Zhang, David Reith, Nuala Helsby, Steve Duffull, James Paxton, Alasdair Millar, Roger Smart, Julie Wilson, Hesham Al-Sallami.

2) Apologies: Ray Morris, Martin Tingle, Nick Holford

3) Minutes of 2009 AGM: These were tabled (in this year's program) and approved.

4) President's report: Presented by Evan Begg and read. Main points to note were as follows.

- Carney connection: This was the first time the meeting was held in conjunction with the Carney Pharmacogenomics symposium. This meant an increase in registrants from 59 in 2009 to 80 this year. However the registrants for Carney only did not pay themselves but were subsidised by Carney. The success of this was to be monitored, and feedback requested.
- Council report: Report from ASCEPT (Australasia) included the establishment of the Specialist Training Committee (STC) and its achievements so far, discussion on SIGs and the strategic plan.
- Sponsorship: It was recognised that the pharmaco/political/ethical scene was making it difficult for us to seek Drug Company sponsorship for the meeting. This has major consequences. Roger Smart of Douglas Pharmaceuticals was thanked for their continued sponsorship of the meeting. This commitment by Douglas, including the regular attendance of Roger over many years, was seen as a different scenario.
- Fred Fastier prize: The Christchurch committee had decided that the Fred Fastier student prize for 2010 would be combined for both orals and posters. There were 8 applicants for orals, and 2 for posters. It was decided that a second prize would also be offered, and that the value would be \$500 for first prize and \$250 for second prize.
- Student travel awards: The committee decided that \$1000 be allocated for 2010. There were 4 applicants, one from Auckland and 3 from Dunedin. The Auckland applicant was granted \$300, and the Dunedin applicants \$200 each.

5) Treasurer's report: Presented by Paul Chin.

- The ASCEPT accounts had balance of \$9598.48 at time of takeover from Dunedin.
- Sponsorship gratefully received from Douglas Pharmaceuticals \$1000
 - Carney Centre for Pharmacogenomics expected to also reimburse ASCEPT NZ for parts of conference costs
- 57 registrants this year to ASCEPT NZ 2010 ASM with subsidised registration for ASCEPT members as well as additional subsidising of registration, conference dinner for student member of ASCEPT. There were four applications for travel grants received and accepted.
- Outgoings exceed income for this conference by around \$4000
- Bank balance, once all invoices settled will be around \$6000.
- The Fred Fastier Prize account had balance of \$11974.78 at time of AGM, with the account earning \$239.87 in interest after tax in the past year. This represents a low interest rate and the plan is to move this to a term deposit with a higher interest rate.

6) Other business:

- Carney connection: Steve Duffull's group perceived that combining with Carney effectively reduced the length of the ASCEPT component from 1.5 to 1 day. This was not intended, but should be factored in to further discussion.
- Representation from different Universities: Steve Duffull noted that there was only one member, and one retiring member from the Pharmacology Department, University of Otago, which was disappointing in view of this being one of the larger departments in NZ. The justification given was that the dates of the meeting clashed with other conferences. Evan Begg pointed out that the dates were chosen based on discussions with both Dunedin and Auckland pharmacology departments, to pre-empt this problem. We need to find out why this meeting is not considered a priority for some departments. For the record, the representation from different groups at this year's conference was: Auckland Pharmacology 11, Auckland other 1, Wellington 3, Christchurch Clinical Pharmacology 13, Christchurch other 22, Dunedin Pharmacy 12, Dunedin Pharmacology 2, Dunedin other 7, Australia 5, Other countries 3.
- Student travel grants: Steve Duffull raised the issue that travel grants preclude non NZ residents. This was generally thought to be not so, and that travel grants could be considered for any person who was a member of ASCEPT and a bona fide student with a department within Australasia. This should be made clear. The granting of travel grants and the amounts involved are at the discretion of the presiding Committee.
- Registration costs: In view of the declining ability to obtain sponsorship for the meeting, it was felt that the price of registration should be raised to reflect and cover true costs. This year the cost of registration was \$150, and the dinner \$75. The true costs were somewhat higher especially for the dinner, for which the true cost (food + drink) was \$105 pp. Students were subsidised further. It was passed unanimously that the cost of registration should be increased, up to \$250 if necessary, and that the dinner and conference should be cost neutral.

1. ASCEPT PLENARY/SESSION 1 – CHAIR: EVAN BEGG

1.1 PLENARY SPEAKER

TAKING THE PISS IN THE CRITICALLY ILL: WHEN $1+1 \neq 0.5$

Carl MJ Kirkpatrick, Centre for Medicine Use and Safety, Monash University, Parkville, VIC

Renal replacement therapy (RRT) is used in ~5% of intensive care unit (ICU) admissions for the treatment of acute renal failure. In ICUs, there are a number of renal replacement therapies utilised including CVVHD, CVVHDF and SLED. The “golden hours” post admission to ICU requires optimal pharmacotherapy and antimicrobial dosing to decrease morbidity and mortality. It is recognised that none of the RRT modalities have clearances approaching a well functioning kidney. The water solubility and protein binding are often used to predict the likely impact of RRT on the pharmacokinetics of each drug. Patients receiving RRT generally receive a similar loading dose, followed by either a lower dose or the same dose with an extended dose interval as a maintenance dose. The aim is to achieve similar concentrations to those observed in healthy patients receiving a normal dose.

Two antimicrobial drugs are used to highlight the importance of understanding the mechanistic pathways involved in the elimination of drugs via the kidney when considering the effect of RRT on pharmacokinetics. Population pharmacokinetic models were developed to describe the impact of RRT on the pharmacokinetics in a ICU population. Monte Carlo simulations were undertaken to evaluate the ability of current guidelines to achieve the desired PK/PD targets i.e. efficacy and/or toxicity. For drug 1, a relatively normal dose with prolonged dosing interval is optimal for efficacy and toxicity, while for the second drug larger doses than healthy patients are required to achieve the PK/PD targets for efficacy. This work highlights that the assumption that all drugs have decreased clearance in RRT is not always true. Indeed further consideration is required to optimise pharmacotherapy in the ICU care setting particularly when RRT is utilised.

1.2

THE PERFORMANCE OF GFR FORMULAE FOR ESTIMATING GENTAMICIN CLEARANCE

Paul KL Chin^{1,2}, Evan J Begg^{1,2}. Department of Clinical Pharmacology, Christchurch Hospital¹, Christchurch, NZ, University of Otago, Christchurch², NZ

Introduction: Gentamicin is a polar, renally cleared drug. Traditionally, the Cockcroft-Gault (CG) formula has been used to predict its clearance in individuals prior to giving the first dose (*a priori* gentamicin clearance). There are aspects of this formula that may be improved, such as the weight descriptor used and how it deals with low plasma creatinine concentrations. The Modification of Diet in Renal Disease (MDRD) formula has emerged as a valuable alternative for estimating glomerular filtration rate, and may also be useful for predicting gentamicin clearance.

Aims: To compare the performance of the CG and MDRD formulae (and three variants of each) for estimating gentamicin clearance using an existing database of individuals with gentamicin clearances calculated from gentamicin concentrations (*a posteriori* gentamicin clearance).

Methods: Comparisons were made using the Pearson’s correlation coefficients (r), the Bland-Altman method for comparison, and chi-squared analyses of the proportions of *a priori* gentamicin clearance estimates within 30% and 50% of the *a posteriori* gentamicin clearance values.

Results: Gentamicin clearance estimates using the eight formulae were generated for 1516 individuals. The formula with the highest r was the CG formula incorporating lean body weight and a minimum plasma creatinine concentration of 0.06 mmol/L ($r = 0.86$). This formula also had the smallest mean bias (-4 mL/min) and narrowest 95% limits of agreement (± 29 mL/min) according to the Bland-Altman analyses. It also had the highest proportion of patients within 30% (0.90) and 50% (0.99) of the *a posteriori* gentamicin clearance values ($P < 0.0001$).

Discussion: The CG formula incorporating lean body weight and a minimum plasma creatinine concentration of 0.06 mmol/L provided the best estimate of gentamicin clearance from this dataset.

Janmahasatian S et al (2005) Clin Pharmacokinet 2005; 44: 1051-65.

1.3

STARTING DOSE, BUT NOT MAXIMUM MAINTENANCE DOSE, IS A RISK FACTOR FOR ALLOPURINOL HYPERSENSITIVITY SYNDROME: A PROPOSED NOMOGRAM FOR SAFE STARTING DOSE OF ALLOPURINOL

Lisa K Stamp^{1,2}, William J Taylor³, Peter B Jones⁴, John L Dockerty⁵, Jill Drake², Chris Frampton¹, Nicola Dalbeth⁶. Department of Medicine, University of Otago – Christchurch¹, Rheumatology and Immunology Department, Christchurch Hospital², Department of Medicine, University of Otago – Wellington³, Department of Medicine, University of Auckland⁴, Dunedin School of Medicine⁵

Introduction: Allopurinol is the most commonly used urate lowering therapy in gout. Allopurinol hypersensitivity syndrome (AHS) is a rare but potentially fatal adverse event. CrCL-based dosing guidelines have been proposed based on the recognition that doses $\geq 300\text{mg/d}$ may be associated with AHS, particularly in patients with renal impairment.

Aims: The aim of this study was to determine the relationship between allopurinol dosing and AHS.

Methods: A retrospective case-control study of patients with gout who developed AHS between January 1998 and September 2010 was undertaken. For each case three controls with gout receiving allopurinol who did not develop AHS were identified. Controls were matched on gender, diuretic use at the time of commencing allopurinol, age ± 10 years, and eGFR. Analysis compared starting dose and maximally achieved dose between cases and controls.

Results: 54 AHS cases and 157 controls were identified. There was an increase in risk of AHS as the starting dose of allopurinol corrected for eGFR increased. For the highest quintile of starting dose/eGFR, the odds ratio was 23.2 ($p < 0.01$). There was no significant difference in the means of the maximum doses of allopurinol ever achieved between cases and controls. Using ROC analysis, starting allopurinol at a dose $\geq 1.5\text{mg}$ allopurinol/eGFR (mg/ml/min) was associated with 90% sensitivity and 37.5% specificity for AHS.

Conclusions: Starting allopurinol at a dose of 1.5mg/eGFR may greatly reduce the risk of AHS. Progressive up-titration of dose to achieve the target serum urate is not associated with an increased risk of AHS.

2. ASCEPT SESSION 2 – CHAIR: JANE VELLA-BRINCAT

2.1

A POPULATION PHARMACOKINETIC MODEL FOR ALLOPURINOL IN GOUT PATIENTS

Dan FB Wright¹, Lisa K Stamp², Rebecca L Roberts^{2,3}, Nick HG Holford⁴ & Stephen B Duffull¹. Departments of Pharmacy¹ and Biochemistry³ Univ of Otago, Dunedin, NZ, Department of Medicine², Univ of Otago, Christchurch, NZ, Department of Pharmacol and Clin Pharmacol⁴, Univ of Auckland, Auckland, NZ

Aims: (1) To develop a population pharmacokinetic (PK) model for allopurinol and its active metabolite, oxypurinol, (2) To explore patient characteristics (covariates) which influence the pharmacokinetics of allopurinol and oxypurinol.

Methods: The data were sourced from a dose escalation study (Stamp *et al* 2011). 45 patients with gout on a stable dose of allopurinol had doses increased by 50-100mg/month until a target serum urate of $< 0.36\text{ mmol/L}$ was achieved. An additional 45 patients were on the CrCL-based dose of allopurinol with $\text{SUA} < 0.36\text{ mmol/L}$. Steady-state allopurinol and oxypurinol plasma concentrations were collected at each clinic visit. The population pharmacokinetics of allopurinol and oxypurinol were estimated using NONMEM[®] v.7.2. Covariates analysed included, creatinine clearance, body weight (total and lean), diuretic use, and aldehyde oxidase (AOX1) and molybdenum cofactor sulfuryase (MOCOS) genotypes.

Results: 884 allopurinol and oxypurinol plasma concentrations were available for analysis. Plasma concentrations below the limit of quantification (LOQ) were retained and treated as censored observations using “Method 3” as described by Beal 2001. The data were best described by a one-compartment parent-metabolite model. We assumed a parent to metabolite conversion of 80%. The population estimates for allopurinol clearance (CL) and volume of distribution (V) were 80 L/h (CV% 54) and 55 L (CV% 33) respectively. The population estimate for oxypurinol CL and V were 0.82 L/h (CV% 66) and 28 L (CV% 6) respectively. CL and V were significantly influenced by lean body weight (allopurinol and oxypurinol) while the CL of oxypurinol was also correlated to creatinine clearance and the concomitant use of diuretics. AOX1 or MOCOS genotypes were not found to significantly affect allopurinol clearance.

Conclusions: A population PK model for allopurinol and oxypurinol was developed. Differences in lean body weight, creatinine clearance and the use of diuretics would be expected to influence the variability in dose requirements between patients.

Stamp LK *et al* (2011). Clin Pharmacol Ther In Press.

Beal SL (2001). J Pharmacokinetic Pharmacodynamic 28:481-504.

2.2

DEVELOPMENT AND APPLICATION OF A PHARMACOKINETIC MODEL FOR THE GLYCATION OF ALBUMIN

Julia Korell¹, Oskar Alskaer^{1,2,*} & Stephen Duffull¹. ¹School of Pharmacy, University of Otago, Dunedin, NZ, ²Department of Pharmaceutical Biosciences, Division of Pharmacokinetics and Drug Therapy, Faculty of Pharmacy, Uppsala University, Sweden. * first author

Introduction: Glycated haemoglobin, HbA1c, is used commonly as a marker for glycaemic control. In patients with chronic kidney disease (CKD) red blood cells (RBCs) are removed faster from the circulation, giving less time for glycation of haemoglobin to occur [1]. Thus, HbA1c concentrations are falsely lowered in these patients. Glycated albumin (GA) has been suggested as an alternative marker of glycaemic control in patients with CKD [2,3] since it is independent of the RBC lifespan and unaffected by CKD. Furthermore, GA has a half-life of only about 20 days and reflects changes in blood glucose concentrations faster than HbA1c.

Aim: The aim of the project was to develop a model that describes the time course of GA in order to assess its potential clinical benefits.

Methods: A model for GA was developed based on data extracted from the literature and modelled using NONMEM[®]. Simulations were carried out in MATLAB[®]. Predictions of the GA model were compared to predictions of clinical benefits from a model for the time course of HbA1c from [4]. GA and HbA1c model predictions were compared to investigate the difference in response to a change in 24h-mean blood glucose concentration when diabetes treatment commences.

Results: The GA model described the literature data well. Between subject variability in GA was larger than for HbA1c. Simulation of a decrease in mean plasma glucose concentrations resulted in a faster change in GA compared to HbA1c.

Discussion: GA could potentially be used as an alternative marker to assess glucose control in diabetic patients with CKD. GA could be used instead of HbA1c as biomarker in phase II clinical trials for novel antidiabetic drugs.

References:

- [1] Viljoen M *et al.* (1991). *Nephron*. 52(2):271-278.
- [2] Inaba M *et al.* (2007). *J. Am. Soc. Nephrol.* 18(3):896-903.
- [3] Peacock T *et al.* (2008). *Kidney Int.* 73(9):1062-1068.
- [4] Kalicki R *et al.* (2009). PAGE 18 Abstr 1677 [www.page-meeting.org/?abstract=1677], St. Petersburg, Russia.

2.3

ESTIMATING FAT-FREE MASS IN CHILDREN

Hesham S Al-Sallami¹, Ailsa Goulding², Rachel Taylor², Andrea Grant², Sheila Williams², & Stephen B Duffull¹. School of Pharmacy, University of Otago, Dunedin, NZ¹, School of Medicine, University of Otago, Dunedin, NZ²

Introduction: Body size correlates with clearance and can be used to scale drug doses. Fat-free mass (FFM) has been proposed to be a better size descriptor than other measures of weight especially in obese subjects. Mathematical models for estimating FFM have been developed in adults (Janmahasatian *et al.*, 2005). There are currently no models available to predict FFM in children.

Aims: To develop a model to quantify fat-free mass in children.

Methods: Two models (M1, M2) were developed to describe FFM in children. M1 was an empirical model that contained all possible factors and was developed in STATA v11. M2 was a simpler mechanistic type model and was developed in NONMEM v7.2. The models were built from an index dataset (496 females and 515 males). M1 was developed to provide the best possible description of the data (aka a positive control). In addition, the adult model (M3) (Janmahasatian *et al.*, 2005) was used as a naive description of the data (aka negative control). The predictive performance of the three models was assessed using root mean squared error (RMSE). A test dataset (90 females and 86 males) was available for external evaluation.

Results: M1 consisted of 9 terms with interactions (age, sex, height, weight, tanner score, and bone mass). M2 was an asymptotic exponential maturation model based only on age. For the index data set, the RMSE for M1, M2 and, M3 were 2.5, 3.4, and 3.9 kg respectively. For the test data set, the RMSE for M1, M2, and M3 were 2.9, 3.4, and 4.1 kg respectively.

Discussion: A maturation model to estimate FFM in children was developed. The model provided a more precise estimate of FFM in children than the adult model.

References:

- Janmahasatian *et al.* *Clin Pharmacokinet* 2005; 44(10): 1051-1065

2.4

POPULATION PHARMACOKINETICS OF GENTAMICIN IN OLDER PEOPLE: THE IMPACT OF FRAILITY

Claire F Johnston^{1,2,3}, Sarah N Hilmer^{1,2}, Andrew J McLachlan^{3,4} & Carl MJ Kirkpatrick⁵. Syd Med School, Univ of Sydney, Sydney NSW¹; Dept of Clin Pharmacol and Ageing, Royal North Shore Hosp, St Leonards, NSW²; Dept of Pharm (Aged Care), Concord RG Hosp, Concord, NSW³; Faculty of Pharm, Univ of Sydney, Sydney, NSW⁴; Faculty of Pharm, Monash Univ, Parkville, VIC⁵.

Introduction: Frailty becomes more prevalent with increasing age and is demonstrated to influence the pharmacokinetics (PK) of certain drugs, like gentamicin (Hilmer *et al*, 2011).

Aims: The primary aim of the study was to estimate the population PK parameters for gentamicin in an older, hospital population, and to evaluate the effect of frailty on gentamicin CL.

Methods: Demographic, dosing and concentration data was available from 31 older patients with a median (range) age 80 (65-96) years, 26% female and 52% frail as measured by the Reported Edmonton Frailty Scale. Gentamicin was administered to 23 patients as prophylaxis for cystoscopy and 8 patients for the treatment of suspected sepsis. The total number of gentamicin concentrations was 74 with a median (range) 2 (1-3) concentrations per patient. NONMEM-7 was used to estimate the population PK of gentamicin.

Results: A one-compartment linear model with Between Subject Variability (BSV) on CL and V best described the data. The inclusion of creatinine clearance (CLCR) calculated using the Cockcroft-Gault equation with Lean Body Weight (LBW) (Janmahasatian *et al.*, 2005) reduced the random component of BSV from 33% to 18%, with a further reduction to 15% with the addition of frailty to the covariate model. For the final covariate model the population estimate of CL for non-frail patients was 6.2L/h per 100ml/min CLCR and 15.1L per 55kg LBW. For frail patients, the population value of CL was reduced by ~20%.

Discussion: Variability in gentamicin CL in older patients can be partly explained by frailty, even after adjusting for CLCR. Frail patients showed ~ 20% decrease in CL compared to non-frail patients, which may represent the need for a decreased dose recommendation for frail older patients when treated with gentamicin.

Hilmer SN *et al* (2011) *BJCP* 71(2):224-231.

Janmahasatian S *et al*(2005) *Clin Pharmacokinet* 44(10):1051-1065.

2.5

EXTENDED-INTERVAL GENTAMICIN DOSING IN ADULT PATIENTS WITH IMPAIRED RENAL FUNCTION – SHOULD THE DOSE INTERVAL BE EXTENDED FURTHER?

Evan J Begg, Sabina M Plajer, Paul KL Chin, Jane WA Vella-Brincat & Pamela Buffery. Dept of Clinical Pharmacology, Christchurch Hospital and University of Otago, Christchurch, NZ

Aims: To examine a large database of patients treated with gentamicin to see how many (%) achieved the target endpoints of EID: peak concentration (C_{max}) > 10 mg/L, trough concentration (C_{min}) < 0.5 mg/L and area under the curve over 24 hours (AUC_{24}) = 70 to 100 mg/L·h. To determine which of patients might benefit from longer dose intervals (36h, 48h). To evaluate nephrotoxicity in this patient group.

Methods: A database of gentamicin dose predictions at Christchurch Hospital over the 14 years 1996-2010 was analysed. Bayesian dose prediction software SeBA-GEN (version 1.1) was used to calculate individual patient pharmacokinetic values. Simple one-compartment pharmacokinetic calculations were used to calculate $t_{1/2}$ thresholds for longer dose intervals. A nephrology database of haemodialysis patients and a laboratory database of serum creatinine concentrations over the same period were combined with the above to assess nephrotoxicity.

Results: Data from 4504 patients (after exclusions) were available. 96% achieved C_{max} > 10mg/L, 82% C_{min} < 0.5mg/L and 51% AUC 70 to 100 mg/L·h. In order to achieve the target endpoints, patients with $t_{1/2}$ 5.4–8.2h (9%) required 36h dosing while patients with $t_{1/2}$ > 8.2 (1% of patients) required 48h dosing. No evidence of gentamicin induced nephrotoxicity was seen.

Conclusions: EID with 24h dosing achieved satisfactory target C_{max} but not C_{min} . Achievement of target C_{min} could be improved by extending the dosing interval beyond 24h in around 10% of the population who have significant renal dysfunction. Nephrotoxicity was very rare in this population, and would likely be even less with the proposed dosing strategy.

3. ASCEPT SESSION 3 – CHAIR: BERIT JENSEN

3.1

DEMONSTRATION OF SYMPTOMATIC AND DISEASE MODIFYING EFFECTS OF LEVODOPA IN PARKINSON'S DISEASE USING THE ELLDOPA STUDY

Bart Ploeger^{1,2} & Nick Holford³. DMPK, AstraZeneca R&D, Södertälje, Sweden Division of Pharmacology¹, Leiden/Amsterdam Center of Drug Research, Leiden University, Netherlands², Department of Pharmacology and Clinical Pharmacology, University of Auckland, NZ³

Introduction: The symptomatic relief of levodopa in Parkinson's disease has been well established, but controversy exists about possible disease modifying effects.

Aims: To confirm the symptomatic and disease modifying effects of levodopa by analysis of the observations in the ELLDOPA study.

Methods: Previously untreated parkinsonian patients were randomized to a levodopa daily dose of 150 mg (n=92), 300 mg (n=88), 600 mg (n=91) or placebo (n=90) for 40 weeks then withdrawal for 2 weeks [1]. Data were analysed using a linear disease progression model and a mixed-effects modeling approach [2]. For the inactive treatment effects a mixture model was applied to distinguish patients who improved (placebo) or worsened (nocebo). A delayed symptomatic effect, resulting in a transient change in the offset of the disease progression curve was combined with an immediate onset disease modifying effect.

Results: The analysis confirmed the combined symptomatic and disease modifying effects of levodopa, as predicted previously using the DATATOP cohort [3]. Approximately 25% of the patients showed a nocebo effect (transient worsening) while the rest had a placebo effect (transient improvement), which is similar to the results of Ma et al. [4] using inactive treatment groups from 3 other studies. For both the nocebo and placebo effects patients with either a fast or slow onset were identified. The disease progression rate was higher in the population that dropped out.

Conclusion: This analysis confirms that levodopa has symptomatic and disease modifying effects.

1. Fahn S, Oakes D et al. Levodopa and the progression of Parkinson's disease. *N Engl J Med.* 2004;351(24):2498-2508.
2. Holford NH, Nutt JG. Interpreting the results of Parkinson's disease clinical trials: Time for a change. *Mov Disord.* 2011;26(4):569-77.
3. Chan PL, Nutt JG, Holford NHG. Levodopa slows progression of Parkinson's disease: external validation by clinical trial simulation. *Pharm Res.* 2007;24(4):791-802.3)
4. Ma SC, Holford NHG. Quantifying Disease Progress with Inactive Treatments in Multiple Parkinson's Disease Trials. PAGANZ 2011

3.2.

MASS BALANCE, METABOLISM AND EXCRETION OF [¹⁴C]ASA404 IN CANCER PATIENTS

Mark McKeage¹, Peter Fong¹, Xian Kang Hong², Jimmy Flarakos², James Mangold², Yancy Du², Chiaki Tanaka² & Horst Schran². ¹The University of Auckland, Auckland, New Zealand; ²Oncology, Novartis Pharmaceuticals Corporation, East Hanover, New Jersey, USA

Introduction: ASA404 is a small molecule flavonoid tumour vascular disrupting agent that has been evaluated as an anticancer therapy in combination with standard chemotherapy.

Aims: This study aimed to assess the mass balance, metabolism and excretion of [¹⁴C]ASA404 in patients with advanced cancer.

Methods: Seven cancer patients were given a single dose of 3000 mg of [¹⁴C]ASA404 by intravenous infusion over 20 minutes, followed by up to six 3-weekly cycles of nonradioactive ASA404 given either alone or combined with docetaxel, carboplatin and/or paclitaxel. Blood, urine and faeces samples were collected to assess ASA404 and total radioactivity pharmacokinetics over a seven day period. Samples were analysed by LC-MS, liquid scintillation counting, HPLC with offline radioactivity detection and LC-MS/MS.

Results: Unchanged ASA404 was the dominant drug-related component in plasma and had a long terminal elimination half life 27.2 hr. Excretion of radioactivity in the urine and faeces accounted for approximately 54% and 33% of the ASA404 dose, respectively. Its major metabolic pathways were the hydroxylation of carbon 6 and glucuronidation of the carboxylic acid. ASA404 was eliminated in urine and faeces mainly in the form of these metabolites rather than unchanged drug.

Discussion: this study shows that ASA404 was the major drug-derived species in the systemic circulation likely to have contributed to its tumour vascular disrupting activity in cancer patients, and that its 6-hydroxy and acyl glucuronide metabolites are the main forms excreted in the urine and faeces.

3.3

IS IT NICE TO MONITOR LITHIUM ROUTINELY?

Andrew McKean¹, Jane Vella-Brincat². Pharmacy Department, Hillmorton Hospital¹, Christchurch, New Zealand, Dept of Clinical Pharmacology, Christchurch Hospital², Christchurch, New Zealand

Introduction: Lithium has a narrow and well described therapeutic range.

Aim: The aim of this study was to evaluate lithium blood concentration monitoring in Canterbury District Health Board (CDHB) and consider whether it meets the UK National Institute for Health and Clinical Excellence standard (in lieu of more local advice).

Methods: Lithium dispensing data for patients within the CDHB boundaries was combined with lithium blood concentrations for the period of 1st July 2009 to 30th June 2010 and the results analysed.

Results: Lithium was prescribed for 1416 patients with a mean daily dose of 507mg per day. 95% of patients in CDHB had had a lithium blood concentration performed at least once during the year. Twenty percent had had four or more lithium blood concentrations analysed. The mean (\pm 95% CI) lithium blood concentration was 0.63 (0.62 to 0.64) mmol/L; the median (interquartile range) was 0.6 (0.43 to 0.80) mmol/L and the range was 0 to 2.8mmol/L. The median (interquartile range) sampling interval was 35 (13-93) days. Sampling was performed approximately every three months (80 to 100 days) in 11 patients (<1%). Of those 56 patients that had a lithium blood concentrations >1.2 mmol/L only seven patients had this repeated within three weeks.

Discussion: In conclusion, lithium blood monitoring at CDHB did not achieve the NICE standard. This is in keeping with a number of other audits conducted of lithium blood monitoring.

3.4

IN SILICO ASSESSMENT OF ERLOTINIB AS A POTENTIAL INHIBITOR OF CYP3A-MEDIATED DRUG CLEARANCE

Ganessan Kichenadasse^{1,2}, Matthew P Doogue¹, Bogda Koczwara², Chris Karapetis², John O Miners¹ & Thomas M Polasek¹. Dept of Clin Pharmacol, Flinders Medical Centre and Flinders University¹, Adelaide, SA, 5042, Dept of Med Oncol², Flinders Medical Centre and Flinders University², Adelaide, SA, 5042

Introduction: Erlotinib, a tyrosine kinase inhibitor of epidermal growth factor receptors (EGFR), is used to treat lung and pancreatic cancers. Its clearance is primarily mediated by CYP3A (70%), making erlotinib susceptible as an 'object' of pharmacokinetic drug-drug interactions (PK-DDIs) (1). *In vitro* data describe erlotinib as a moderately potent mechanism-based inactivator (MBI) of CYP3A (2), indicating that it may increase the concentration of drugs metabolised by CYP3A.

Aim: To assess erlotinib as a potential inhibitor of CYP3A-mediated drug clearance using SimcypTM.

Methods: A previously published erlotinib profile (1) was modified in Simcyp (V9.1) to incorporate the *in vitro* inactivation kinetic parameters for CYP3A (2). Simulations were run for single and multiple doses of erlotinib in a virtual population of 1000 healthy Caucasians. Two interaction studies were simulated in the same cohort to assess the effects of single dose (Trial I) and multiple doses (Trial II) of oral erlotinib on the clearance of oral midazolam (CYP3A 'probe').

Results: The simulated AUC for single dose erlotinib was consistent with published PK data. Multiple-dose erlotinib simulations incorporating MBI kinetic data were consistent with clinical PK study results. When single dose erlotinib was given together with midazolam, the mean simulated AUC of midazolam increased 3-fold (Trial I), while 7 days of erlotinib increased the AUC 13-fold (Trial II). These results contradict a small clinical PK interaction study in cancer patients (24-30% decrease in midazolam AUC) (5).

Discussion: Our profile for erlotinib improves the previous physiological-based PK model (1). Erlotinib is a competitive inhibitor, allosteric modulator and inducer of CYP3A enzymes (4, 5) but *in vitro* data to quantify these interactions are unavailable. Such complex interactions with CYP3A may explain the observed disparity between the interaction studies conducted *in silico* and *in vivo*. Further *in vitro* data are needed to develop a sufficiently robust *in silico* model to reduce inaccurate predictions of PK-DDI potential.

References:

1. Rakhit A et al. *Eur J Clin Pharmacol* 2008; 64: 31- 41.
2. Li J et al. *Clin Cancer Res.* 2007; 13: 3731-37.
3. Calvert H et al. *J Clin Oncol.* 2005; 23 (No. 16S): 3076.
4. Harmsen S et al. *Cancer Chemother Pharmacol.* 2009; 64: 35- 43.
5. Liu Y et al. *Drug Metab Dispos.* 2010; 38: 32-9.

3.5

FONDAPARINUX: NOT SO SIMPLE RENAL ELIMINATION

Paul J Zufferey & Stephen B Duffull. School of Pharmacy, University of Otago, Dunedin, NZ

Introduction: Fondaparinux is a synthetic antithrombotic agent with specific anti-factor Xa activity. After a subcutaneous dose, non-compartmental pharmacokinetic analysis indicates that fondaparinux has an absolute bioavailability of 100 % with linear pharmacokinetics in the range of 2 to 8 mg. Fondaparinux is highly bound to antithrombin III (fraction unbound = 3%) and is almost completely excreted unchanged in the urine (1). POP-A-RIX and PROPICE are two cohort studies of orthopaedic patients treated with fondaparinux for venous thromboembolism prophylaxis.

Aim: to develop a population pharmacokinetic (PK) model to characterize and quantify covariates that may explain different dosing requirements of fondaparinux. This study focused on the relationship between clearance of fondaparinux and mechanisms of renal elimination.

Methods: 3439 plasma anti-Xa activities were obtained from 999 patients (POP-A-RIX, fondaparinux 2.5 mg per day) and 444 patients (PROPICE, fondaparinux 1.5 mg per day in patients with moderate renal impairment) for a population PK analysis using NONMEM VII software.

Results: A two compartmental model with first order administration best described fondaparinux PK. Clearance exceeded the contribution of glomerular filtration suggesting the occurrence of a renal secretion process. For secretion the data support either a nonlinear saturable process or a linear process with two polymorphisms (57%:43%) with lean body weight as a covariate for each subpopulation. Predicted normal weight significantly explained the variability for apparent volume of distribution of the central compartment.

Discussion: A renal secretion process appears to be the main mechanism of fondaparinux clearance. As previous analysis suggested that PK of fondaparinux was linear in a large range of dose, a saturable process for secretion is unlikely. The different values of clearance could be due to environmental polymorphism associated with drug interactions or polymorphism or as a yet undetermined genetic polymorphism of this secretion pathway. Factors to help predict patients with low or high secretion were not able to be identified.

(1) Donat F et al. Clin Pharmacokinet 2002; 41 Suppl. 2 : 1-9

4. ASCEPT SESSION 4 – CHAIR: PAUL CHIN

4.1

THE CURRENT FUNDING MODEL LEAVES PHARMACISTS CAUGHT IN THE STRUGGLE BETWEEN JUSTICE AND NON-MALEFICENCE

Stephen Duffull, Ben Emery, Ben Robertson, Presant Singh & Everard Tolerton. The School of Pharmacy, University of Otago, Dunedin, NZ

Introduction: Dispensing errors in community pharmacies are an unavoidable problem largely due to human error. Pharmacists are funded on a flat fee for dispensing service. We suggest that for the error rate to approach zero the time taken to dispense each prescription would approach infinity. The principles of justice (access to healthcare & a financially viable pharmacy) and non-maleficence (do no harm) are naturally opposed in this model. We propose the existence of two hypothetical populations of pharmacists; those that promote the principle of non-maleficence (slow dispensers) and those that promote the principle of justice (rapid dispensers).

Aim: The aim of this work is to explore whether both populations can co-exist within the current funding model.

Methods: The interaction between these two populations was explored by simulation within the context of a repeated non-sum game using game theory. The populations were characterised by rapid pharmacists with an error rate of 10% and careful slower pharmacists with an error rate of 1%. Patient turnover period, patient preference for speed of service, and a patient not returning to a pharmacy where an error occurred were included as variables. The game was run to assess the presence of a stable equilibrium (Nash equilibrium) for each population. The game was also run to assess for evolutionary stable strategies.

Results: The results illustrate that both types of populations of pharmacists could reach a stable equilibrium independently; these stable states were altered by changing patient preference for speed of service and patient turnover. Populations solely consisting of either careful or rapid pharmacists were not stable strategies.

Conclusion: A strategy consisting solely of non-maleficence or justice is not stable. Under the current funding model, striving for no dispensing errors is not necessarily in the best interests of pharmacists or patients.

4.2

GENERAL PRACTITIONERS' PERCEPTIONS OF PHARMACIST PRESCRIBING IN NEW ZEALAND

Ernieda Hatah¹, Rhiannon Braund¹, Stephen Duffull¹ & June Tordoff¹. School of Pharmacy, Univ of Otago, Dunedin, NZ¹

Introduction: Pharmacists in New Zealand are likely to be awarded prescribing rights in the near future. It is intended that this is a collaborative prescribing role which for community pharmacists would mean close liaison with general practitioners (GPs) (PCNZ, 2009). It is unknown how GPs perceived this proposed role.

Aims: To evaluate perceptions of general practitioners about pharmacist prescribing.

Methods: Qualitative, face to face semi-structured interviews of GPs were undertaken. The cohort of GPs included those with different years of practice and included GPs who had experience in areas where some patients had undergone medication use reviews (MURs) by community pharmacists. Data were thematically analysed using constant comparison and NVivo 8 software.

Results: We interviewed 18 GPs from two localities in New Zealand. GPs perceived pharmacist prescribing to be acceptable with appropriate controls for a limited range of simple medications and conditions and following appropriate training. Repeat prescribing by pharmacist was viewed as having the potential for discontinuity of care and interfering with GP-patient relationships. However some GPs agreed with repeat prescribing for limited conditions, but not for patients with chronic disease. There were mixed feeling about pharmacist prescribing warfarin. GPs perceived this might reduce GPs' and nurses' workload but several GPs preferred nurses to provide this service. Similar themes were found between GPs with difference years of practice and experience.

Discussion: GPs acceptance of pharmacist prescribing appears to depend on how they understand the service and its impact on GP practice and patients. GPs in this study were in some agreement with a limited prescribing model for pharmacists with appropriate controls and training, and in close collaboration with a doctor.

4.3

THE PHARMACOKINETICS OF THE ANTIEMETIC CYCLIZINE, AND HOW IT FEELS TO BE SUBJECTED TO BLOOD SAMPLE ORIENTATED RESEARCH, IN PALLIATIVE CARE

Jane W A Vella-Brincat^{1,2}, Evan J Begg^{1,3}, Berit P Jensen³, Paul K L Chin¹, Rebecca L Roberts^{3,4}, Mary Fairhall², Sandy (A D) Macleod^{2,5}, Kate Reid^{5,6} & Jackie Walker². ¹Clinical Pharmacology, Christchurch Hospital, Christchurch, NZ, ²Nurse Maude Hospice, Christchurch, NZ, ³Department of Medicine, University of Otago, Christchurch, NZ, ⁴Department of Biochemistry, University of Otago, Dunedin, NZ, ⁵Health Science Centre, University of Canterbury, Christchurch, NZ, ⁶Hospice Palliative Care Education, Christchurch, NZ

Introduction: Cyclizine, an antihistaminic antiemetic, is commonly used in palliative care. Its pharmacokinetics have been poorly studied but its metabolic pathway may involve the Cytochrome P450 2D6 (CYP2D6) enzyme. If this is the case the metabolic ratio of cyclizine to norcyclizine may vary between patients according to their CYP2D6 genotype. The ethics of the involvement of palliative care patients in research is often questioned as it may be detrimental to the quality of life of their final days.

Aims: To deduce the pharmacokinetics of cyclizine, relate its metabolic ratio to CYP2D6 genotype and qualitatively explore, describe and conceptualise the impact of research involving blood sampling on palliative care patients.

Methods: Ten palliative patients initiated on continuous cyclizine subcutaneous infusions had blood samples taken during the approach to steady state which were analysed for cyclizine and norcyclizine concentrations and CYP2D6 genetics. Each patient was invited to describe their experiences of being involved in research. Semi-structured interviews were recorded and analysed utilising the methods of Van Manen and Graneheim and Lundman.

Results: The median (interquartile [iq] range) cyclizine half life ($t_{1/2}$), volume of distribution (Vd) and clearance (CL) were 13 (7-48) hours, 23 (12-30) L/kg and 15 (11-26) mL/min/kg respectively. The median overall metabolic ratio at steady state was 4.9 (3.8-9.2) and did vary with CYP2D6 genotype ($p=0.02$). Six out of 10 patients in the cyclizine study were able to be interviewed. Two main themes emerged: altruism – participation for the benefit others, and stoicism - accepting frequent blood sampling.

Conclusion: Palliative patients have similar cyclizine pharmacokinetics to those reported in other patient groups. Cyclizine metabolism to norcyclizine may include CYP2D6 as the metabolic ratio did vary with CYP2D6 genotype. Participating in such research study was largely for altruistic reasons and stoicism was also exhibited.

4.4

AN AUDIT OF THE ANTIMICROBIAL GUIDELINE FOR THE TREATMENT OF ACUTE EPIGLOTTITIS IN ADULTS

Melanie R Gamble, Jane Vella-Brincat. Dept of Clin Pharmacol, Christchurch Hosp, NZ.

Introduction: The World Health Organisation recommends that antimicrobial guidelines are developed and audited to promote rational drug prescribing and minimise the emergence of antimicrobial resistance. The Canterbury District Health Board (CDHB) acute epiglottitis antimicrobial guideline recommends iv ceftriaxone followed by oral cefuroxime or oral amoxicillin/clavulanate.

Aims: To evaluate antimicrobial use in the treatment of acute epiglottitis in adults, and audit prescribing adherence with the antimicrobial guideline.

Methods: Medical records were obtained for patients with a discharge diagnosis of acute epiglottitis at Christchurch Hospital between November 2008 and October 2010. Data collected included: patient demographics, antimicrobials used, and microbiology results. Data was collated and analysed using a Microsoft Access™ database.

Results: Eleven patients were identified, with a median age (range) of 47 years (37-64). Ten patients received antimicrobials. Three patients were given initial iv therapy with ceftriaxone, the guideline drug of choice, and two of these also received oral therapy compliant with the guideline (cefuroxime or amoxicillin/clavulanate). The third was given oral cefaclor. Initial therapy with iv amoxicillin/clavulanate, followed by oral amoxicillin/clavulanate was prescribed in five patients. One patient received iv benzylpenicillin followed by oral phenoxymethylpenicillin, and one patient received only oral roxithromycin.

Discussion: Total adherence to the guidelines was poor; only two patients received the recommended antimicrobials for both iv and oral therapy. Adherence to iv ceftriaxone was low, with the majority of patients receiving iv amoxicillin/clavulanate. Adherence was better for oral therapy, where the guidelines were followed in seven cases, six of which received oral amoxicillin/clavulanate. Rather than promoting adherence, the Antimicrobial Guidelines Committee decided that the guidelines should be changed to reflect current prescribing practice and local antimicrobial sensitivities. The audit cycle has been completed by alteration of the guideline to recommend only amoxicillin/clavulanate first line for both initial iv and oral therapy.

4.5

A RETROSPECTIVE PHARMACOKINETIC REVIEW OF THE BUSULPHAN MONITORING SERVICE AT CHRISTCHURCH HOSPITAL

Pamela Buffery¹, Evan Begg¹, Murray Barclay¹ Grant Moore².¹Dept of Clinical Pharmacology, Christchurch Hospital, Christchurch, NZ, ²Toxicology, Canterbury Health Labs, Christchurch, NZ

Introduction: Busulphan is used with other chemotherapy agents for bone marrow conditioning prior to stem cell grafting. It has a narrow therapeutic index necessitating pharmacokinetic monitoring. Such a service is provided by Christchurch Hospital for busulphan patients from all over New Zealand. The results, since 1998, are recorded in a database.

Aims: To examine the busulphan database and analyse the pharmacokinetics of this group of patients (oral and intravenous), to see how many required dose adjustments and to compare predicted versus actual concentrations where repeat assaying has occurred.

Methods: Four or five blood samples are taken from each patient at 30, 60, 90 and 360 minutes for oral busulphan and at 120, 150, 180, 360 and 480 minutes for IV. Concentrations are measured on an Agilent 6890N gas chromatograph using an electron capture detector. The results are recorded on Excel spreadsheet, and the AUC calculated and compared to the target range. The dose is adjusted by linear approximation. Follow-up concentrations occur in some patients.

Results: From 1998 to August 2011 data on 150 patients (266 sample sets) has been recorded, with 63% of patients having two or more sample sets. Following the first dose, 29%, 34% and 27% of patients had a calculated AUC above, within and below the target range, respectively. 9% of patients had an AUC that was not calculable (all oral). 36% were recommended for dose adjustment. Of those who had further monitoring, 22%, 35% and 35% achieved an AUC above, within and below the target range, respectively. Approximately 7% had an AUC that was not calculable.

Discussion: A third of patients had an elevated AUC and, therefore, increased risk of veno-occlusive disease. The busulphan monitoring service improved the number of patients that achieved the target AUC. However, a significant number of patients required further dose adjustment.

5. ASCEPT SESSION 5 - CHAIR: CARL KIRKPATRICK

5.1

A HIGH-THROUGHPUT SCREEN FOR NOVEL DRUGS ACTING ON THE $\alpha 2\beta 2\gamma 1$ GABA_A RECEPTOR

Christine L Dixon¹, Frank Fontaine², Robert J Capon², Pankaj Sah¹ & Joseph W Lynch^{1,3}. Queensland Brain Institute, University of Queensland, Brisbane, QLD¹. Institute for Molecular Biosciences, University of Queensland, Brisbane, QLD², School of Biomedical Sciences, University of Queensland, Brisbane, QLD³

Introduction: GABA_A receptors containing $\gamma 1$ subunits are highly expressed in parts of the brain involved in anxiety and we wish to understand their physiological role. However, we lack the selective probes to separate $\gamma 1$ -containing receptors from the better-studied $\gamma 2$ -containing receptors.

Aims: To identify selective blockers of $\gamma 1$ -containing GABA_A receptors.

Methods: Our screen utilised an iodide-quenchable yellow fluorescent protein (YFP-I152L). This was co-expressed with GABA_A receptor subunits in HEK AD-293 cells, in 384-well plates. Cells were imaged automatically before and after the addition of an iodide Ringers solution, which contained drugs. When GABA_A receptor channels were opened by the addition of GABA, iodide influx was visualised as quench of the YFP. A sample was considered an antagonist if it decreased the fluorescence quench to below that caused by GABA alone, by at least 3 standard deviations on at least 2 replicate screens.

Results: We tested a library of 2688 butanol fractions taken from marine organisms, each fraction containing dozens of compounds, for antagonist activity at $\alpha 2\beta 2\gamma 1$ GABA_A receptors. Of these, 13 fractions showed reproducible antagonist activity. From these fractions, 23 promising compounds were purified and tested for selectivity on the same platform. 3 of these compounds showed strong reproducible antagonism at $\alpha 2\beta 2\gamma 1$ receptors, with reduced activity at $\alpha 2\beta 2$ and $\alpha 2\beta 2\gamma 2$.

Discussion: So far we have 3 possible leads for selective antagonists at $\alpha 2\beta 2\gamma 1$ GABA_A receptors. We intend to use these as a starting point for structure-function analyses, which we hope will result in potent selective drugs for use in brain slice and behavioural studies.

5.2

DEVELOPMENT OF PERFORIN INHIBITORS AS PHARMACOLOGICAL AGENTS

Matthew Bull¹, Nuala Helsby^{1,2}, Julie Spicer¹ & Bill Denny¹. Auckland Cancer Society Research Centre, Univ of Auckland, Auckland, NZ¹, Molecular Medicine & Pathology, Univ of Auckland, Auckland, NZ²

Introduction: Perforin is a vital component of the cytotoxic response pathways of the immune system, and is implicated in the pathology of cerebral malaria and graft-versus-host disease. Inhibition of perforin may be a novel way to suppress immune destruction of normal tissues in these diseases.

Aims: To investigate the pharmacology of a number of potential inhibitor compounds synthesised as part of a drug development program [1].

Methods: A series of 18 analogues was studied for their ability to bind to perforin, using surface plasmon resonance (SPR) spectroscopy. A LC-MS-QQQ assay to detect the compounds was developed and validated and their plasma pharmacokinetics in mice was determined following an i.v. dose.

Results: The ability to bind to immobilised perforin under various conditions was determined by SPR. The compounds had binding affinities between K_D 0.414±0.5 and 7.085±3.3µmol/l, with a R_{max} of 1.5±0.3 to 6.9±0.84RU, although several were too insoluble to generate sufficient data. The rank order of relative binding compares well to *in vitro* inhibitory ability. A 5mg/kg i.v. dose of 16 compounds gave maximum concentrations between 692.8-10034.8 ng/ml with a similar 20 fold range in AUC. The plasma half-life was similar for most compounds ($T_{1/2}$ =1.0-2.4h). The two compounds with the highest C_{max} also recorded the lowest V_D .

Discussion: This preliminary data indicates that these new chemical entities have suitable perforin binding activity and also a sufficient plasma pharmacokinetic profile to continue development as potential drug candidates. Work to establish a) the disposition of these possible drug candidates with a focus on concentrations achieved in blood cells, b) the *in vivo* and *in vitro* metabolic profile and c) the effect of higher doses and multiple administrations on plasma concentrations is ongoing.

[1] Spicer, J.A., Huttunen, K.M., Lyons, D.M., Trapani, J.A., Smyth, M.J., Denny, W.A. Compounds, preparations and uses thereof. WO 2011075784 A1 (166pp), published 30th June 2011.

5.3

OXALIPLATIN TRANSPORT MEDIATED BY ORGANIC CATION/CARNITINE TRANSPORTERS OCTN1 AND OCTN2 IN OVER-EXPRESSING HEK293 CELLS AND RAT DORSAL ROOT GANGLION NEURONS

Nancy N Jong^{1,2}, Takeo Nakanishi², Johnson J Liu¹, Ikumi Tamai² & Mark J McKeage¹. ¹Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ, ²Department of Membrane Transport and Biopharmaceutics, Faculty of Pharmacy, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Japan

Introduction: The organic cation/carnitine transporters OCTN1 and OCTN2 are related to other organic cation transporters (OCT1, OCT2 and OCT3) known for transporting oxaliplatin, an anticancer drug with dose-limiting neurotoxicity. In this study we sought to determine whether OCTN1 and OCTN2 also transported oxaliplatin, and to characterise their functional expression and contributions to its neuronal accumulation and neurotoxicity in dorsal root ganglion (DRG) neurons, relative to that of OCTs.

Methods: [¹⁴C] Oxaliplatin uptake, platinum accumulation and cytotoxicity were determined in OCTN-over-expressing HEK293 cells and primary cultures of rat DRG neurons. Levels of mRNA, protein and functional activities of rOctns and rOcts in rat DRG tissue and primary cultures were characterised using RT-PCR, Western blotting and uptake of model OCT/OCTN substrates, including [³H] MPP⁺ (OCT1-3), [¹⁴C] TEA⁺ (OCT1-3; OCTN1/2), [³H] ergothioneine (OCTN1) and [³H] L-carnitine (OCTN2).

Results: HEK293 cells over-expressing rOctn1, rOctn2, hOCTN1 and hOCTN2 showed increased uptake and cytotoxicity of oxaliplatin compared to mock-transfected HEK293 controls and it was inhibited by ergothioneine and L-carnitine. The uptake of ergothioneine mediated by OCTN1, and L-carnitine mediated by OCTN2, was decreased during oxaliplatin exposure. OCTN1 and OCTN2 mRNA was readily detected in rat DRG tissue and they were functionally active in cultured rat DRG neurons, more so than OCT1, OCT2 or OCT3. DRG neuronal accumulation of [¹⁴C] Oxaliplatin and platinum during oxaliplatin exposure depended upon time, concentration, temperature and sodium, and was inhibited by ergothioneine, to a lesser extent by L-carnitine but not by MPP⁺. Loss of DRG neuronal viability during oxaliplatin exposure was inhibited by ergothioneine but not by L-carnitine or MPP⁺.

Discussion: OCTN1 and OCTN2 both transport oxaliplatin and are functionally expressed by DRG neurons. OCTN1-mediated transport of oxaliplatin appears to contribute to its neuronal accumulation and treatment-limiting neurotoxicity more so than OCTN2 or OCTs.

5.4

KETOTIFEN UPTAKE BY RAT BRAIN ENDOTHELIAL (RBE4) CELLS IS STEREOSELECTIVE

Feifei Feng, Lin Yang, J Paul Fawcett, Hu Zhang & Ian G Tucker. School of Pharmacy, University of Otago, Dunedin, NZ

Introduction: Some first generation cationic H₁-antagonists appear to be transported by an unidentified influx transporter (Ishiguro, et al., 2004; Suzuki, et al., 2010). Ketotifen shows significant inhibition of this transporter suggesting it may also act as a substrate of this transporter.

Aims: To characterize the stereoselectivity of the unidentified transporter using ketotifen as a model cationic H₁ antagonist and RBE4 cells as an in vitro model.

Methods: Ketotifen enantiomers (0.2-100 μM) were incubated with RBE4 cells in 12-well plates for 2 min at 37 °C. Cells were washed 3 times with ice-cold PBS to terminate uptake after which they were lysed using 0.5 ml Milli-Q water and homogenised for 30 s by sonication. Homogenates were then analysed for ketotifen by HPLC and concentrations normalised for protein content measured using the BCA protein assay. Cytotoxicity of ketotifen enantiomers to RBE4 cells was also determined using the MTS assay. Results were analysed and fitted to a mathematical model combining passive and active (Michaelis-Menten) transport using Graphpad Prism 5.

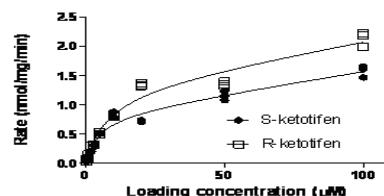
Results: Ketotifen enantiomers were shown to be non-cytotoxic to RBE4 cells in the concentration range used for transport studies. S-Ketotifen and R-etotifen have similar passive diffusion rate constants (0.0075 and 0.0083 ml/mg protein/min respectively) but R-ketotifen ($V_{max}=1.34$ nmol/mg protein/min, $K_m=8.21$ μM) undergoes active uptake twice as fast as S-ketotifen ($V_{max}=0.856$ nmol/mg protein/min, $K_m=4.83$ μM) (see Figure).

Discussion: The uptake of ketotifen enantiomers into RBE4 cells occurs by a combination of active transport and passive diffusion. The active transport is stereoselective and favours R-ketotifen over S-ketotifen. Further studies are ongoing to elucidate the mechanism of uptake.

Ishiguro, N. et al. (2004). *Drug Metab Dispos*, 32:519-524.

Suzuki, T. et al. (2010). *Biopharm Drug Dispos*, 31:243-252.

Acknowledgement: NZ Pharmacy Education and Research Foundation for funding.



5.5

FORENSIC INVESTIGATIONS OF A PSYCHOTROPIC DRUG USING MAGGOTS IN A BRAIN MODEL

Sarah K Bushby¹, Nicky Thomas¹, Carolyn V Coulter¹ & Jules Kieser^{2,3}. Dept of Pharmacy, Univ of Otago, NZ¹, Dept of Dentistry, Univ of Otago, NZ², Sir John Welsh Research Institute, Univ of Otago, NZ³

Introduction: Toxicological analysis of post-mortem tissue can confirm whether psychotropic drugs were implicated in death. Alternative toxicological specimens, such as maggots (*Lucilia sericata*) are used when traditional tissue or blood samples are not available for toxicological analysis. Maggots have a high tolerance to certain toxins, and can grow on tissue spiked with a dose of amitriptyline (Sadler DW et al, 1997_a) or morphine (Hédoin V et al, 2001) lethal to humans. However, it is not possible to predict how a drug will be handled by maggots, with barbiturates often resulting in significant larvae mortality (Sadler DW, et al 1997_b).

Aim: The aim of this project was to develop a method suitable for the detection and quantification of methylphenidate within a maggot matrix, for future applications *in-vivo* toxicological experiments.

Methods: Second-instar *Lucilia sericata* larvae were reared for four days on pig brain spiked with 2 mg methylphenidate per gram of brain tissue. The maggots were washed and stored at -80°C until analysis. Following liquid- liquid extraction of methylphenidate from the maggots the drug was detected either by HPLC or LC-MSMS.

Results: Maggots showed a high tolerance for methylphenidate and can grow on tissue containing methylphenidate levels 5 times greater than the LD₅₀ of methylphenidate in rats. Following extraction of methylphenidate from maggots, the LOQ was 5 µg/ml using HPLC. A more sensitive method using LC-MSMS was then developed, where the LOQ was 150 ng/ml with minimal matrix effect (104 ± 0.43 %).

Discussion: The current study suggests that maggots are suitable alternative specimens for the detection and quantification of psychotropic compounds such as methylphenidate. The newly developed LC-MSMS assay may have potential application in future forensic and toxicological investigations.

Sadler DW et al (1997_a) Am J Foren Med Path 18(4) 397-403

Hédoin V et al (2001) J Foren Sci 46(1) 12-14

Sadler DW et al (1997_b) J Foren Sci 42(3) 481-485

5.6

BIOMARKERS OF AGING TO PREDICT DRUG CLEARANCE IN THE ELDERLY - A PILOT STUDY

Chakradhar V Lagishetty¹, Carolyn V Coulter¹ & Stephen B Duffull¹. School of Pharmacy¹, University of Otago, Dunedin, NZ

Introduction: The Buttler & Begg hypothesis states (free) drug clearance decreases with increasing chronological age (CA) [Buttler et al, 2008]. However, people appear to age at different rates and CA does not account for differences in physiology or pathology which are attributed to biological aging. We propose that a method exists for estimating biological age (BA) that incorporates differences in aging processes. Telomeres have been proposed as a marker of BA [Aviv et al, 2006], these are present at the ends of chromosomes and shorten during each progressive cell division and as a result of other factors e.g., oxidative stress and inflammation. Additionally, the single nucleotide polymorphism (SNP) at rs12696304 is associated with changes in telomere length (TL).

Aim: To develop and evaluate an assay to measure both TL and SNP rs12696304.

Methods: TL and SNP were determined in this pilot study in both young (n=10) and middle aged (n=10) healthy volunteers. DNA was extracted from a blood sample and analysed by qPCR assay for TL and by genotyping for SNP. Change in TL vs age and SNP were compared to literature values.

Results: Both TL and SNP could be accurately characterized. The rate of TL change (-0.0038 per year) was close to that reported previously (-0.005 per year). The median values of TL for middle aged were GC (0.81) and CC (0.92) i.e., in terms of base pairs, GC had 210 fewer base pairs compared to CC.

Discussion: We implemented assays for both TL and SNP. Changes in TL were consistent with literature findings. We intend to initiate a study to assess the influence of these factors on gentamicin clearance.

Buttler et al (2008) Clin Pharmacokinet 47(5):297-321.

Aviv et al (2006) J Gerontol A Biol Sci Med Sci. 61(8):871-873.

6. CARNEY PLENARY/SESSION 1 - CHAIR: MARTIN KENNEDY

6.1 PLENARY SPEAKER

TRANSLATING PHARMACOGENETICS INTO THE CLINIC

Elizabeth J. Phillips, Institute for Immunology and Infectious Diseases, Murdoch University, Perth, Australia

Over the last decade there has been an explosion of discoveries and publications in the fields of pharmacogenomics and pharmacogenetics. Although few of these have traversed the T1àT4 translational paradigm from discovery to application of a test into clinical practice, many of these have been invaluable in their own right by furthering our understanding of disease pathogenesis, and offering the promise for the development of new drug targets. The likelihood that a pharmacogenetic test will make its way into routine clinical practice is driven by a number of factors including characteristics of the drug and the availability of therapeutic alternatives, attributes of the test itself, nature of the drug toxicity, having an environment or individual to champion the test, the ability to generate high levels of evidence to support the clinical utility and cost-effectiveness of the test, the development of appropriate laboratory support, infrastructure and quality assurance, and the design and implementation of appropriate clinical systems. Key examples will be discussed highlighting the discovery and translation of HLA-B*5701 as a screening test used in routine HIV clinical practice to prevent abacavir hypersensitivity syndrome.

6.2

GWAS, G WHIZZ - AN IGNORANT APPROACH TO GENETICS?

Richard Gearry, Department of Medicine, University of Otago, Christchurch, NZ

6.3

LC-MS IN CLINICAL PHARMACOLOGY AND TOXICOLOGY – FROM NICHE PROJECTS TO ROUTINE SCREENING

Berit P Jensen¹, Mei Zhang¹, Grant Moore², Evan J Begg¹; ¹Dept of Medicine, Univ. of Otago, Christchurch, NZ, ²Toxicology, Canterbury Health Labs, Christchurch, NZ

Introduction: Over the past decade Liquid Chromatography-Mass Spectrometry (LC-MS) has evolved from being an expert-only analytical technique to one that is now available in most laboratories that analyse for drugs or drug-related compounds. Most pharmacologists - clinical as well as experimental - will come across data generated via LC-MS, even if they are actively involved in LC-MS analysis themselves.

Aims: The aim of this talk is to give an overview of the LC-MS technology. The basic terms will be explained and examples given of the use of LC-MS in a variety of settings relevant to pharmacology.

Discussion: The technology, while good for routine screening, lends itself to niche projects as it is relatively easy to set up and validate a new LC-MS assay. Features of the technology will be reviewed, which demonstrates why LC-MS has become so successful. Pitfalls and what to look out for, particularly when reviewing literature from a pharmacology perspective, will also be discussed.

7. CARNEY SESSION 2 - CHAIR: REBECCA ROBERTS

7.1

IDENTIFICATION OF MULTIPLE SCLEROSIS PATIENTS WHO ARE NON-RESPONSIVE TO THERAPEUTIC IFN β : AN ASSAY TO ALLOW MORE INFORMED TREATMENT OPTIONS

Hannah L Kennedy¹, Heather Barnes¹, Jane Eagle², Deborah Maon², Peter George¹ & Andrew Fellowes¹. ¹Dept of Molecular Pathology Chch Hospital, Chch, NZ; Dept of Neurology, Chch Hospital, Chch, NZ²

Introduction: Relapsing-Remitting Multiple Sclerosis (RRMS) is the most prevalent form of Multiple Sclerosis. Interferon beta (IFN β) is the first-line treatment however therapeutic effect is reduced in patients who develop neutralising antibodies (NABs). Direct assays for NABs are not readily available. Recent studies have shown that expression levels of IFN β responsive genes are useful biomarkers of NABs. In particular, induction of the MX1 gene is reduced or absent in the blood of NAB positive individuals (McKay et al., 2006).

Aims: To describe the development of a clinical assay of MX1 expression.

Methods: Peripheral blood was collected up to 12 hours post IFN β injection using PAX-Gene RNA blood tubes. A single tube quantitative RT-PCR using LNA probes (Universal Probe Library, Roche) was employed to detect MX1 gene expression relative to expression of the reference gene GAPDH.

Results: A validation study of 17 MS patient samples and 19 interferon-naïve controls showed a significant difference in MX1 expression (unpaired t-test $p=0.0001$). Mean MX1 expression was 22.19 for IFN β -naïve samples (sd 13.34) and 1.594 (sd 0.8576) for MS patient samples. ROC analysis gave an optimal cut point of 3.73 with sensitivity of 100% and specificity of 95.2%. Two patient samples reproducibly exhibited MX1 expression within the range of the IFN β -naïve group (as would be expected of a Nab positive sample). Confirmation that these patients had developed NABs was achieved by the Cytopathic effect assay (CPE).

Discussion: Our data indicates that this assay can accurately discriminate between NAB positive and NAB negative MS patients. An appropriate threshold of NAB positive MxA expression was determined, and an expression limit of NAB negativity is being examined. An equivocal range will be set between these two limits. Patient recruitment is ongoing and this assay is now available for routine use.

MCKAY, F., SCHIBECI, S., HEARD, R., STEWART, G. & BOOTH, D. (2006) Analysis of neutralizing antibodies to therapeutic interferon-beta in multiple sclerosis patients: a comparison of three methods in a large Australasian cohort. *J Immunol Methods*, 310, 20-9.

7.2

DETERMINATION OF 6-THIOGUANINE NUCLEOTIDES AND 6-METHYLMERCAPTOPYRINE NUCLEOTIDES IN HUMAN RED BLOOD CELLS BY LC-MS/MS

Mei Zhang¹, Grant A Moore², Murray L Barclay¹ & Evan J Begg¹. ¹Department of Medicine, University of Otago-Christchurch, New Zealand, ²Toxicology, Canterbury Health Laboratories, Christchurch, New Zealand

Introduction: Azathioprine (AZA) and 6-mercaptopurine (6-MP) are immunomodulators used widely in the therapy of autoimmune diseases. 6-Thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine nucleotides (6-MMPN) are two important metabolites of AZA and 6-MP, with 6-TGN having immunosuppressive and bone marrow toxicity effects, and 6-MMPN exhibiting hepatotoxicity. Concentration monitoring of 6-TGN and 6-MMPN in red blood cells (RBCs) helps to optimise efficacy and reduce the risk of toxicity.

Aims: To develop and validate a simple, sensitive and accurate LC-MS/MS assay for RBC 6-TGN and 6-MMPN monitoring.

Methods: After proteins were precipitated with perchloric acid, 6-TGN and 6-MMPN were hydrolysed at 120°C to the free 6-TG and 6-MMP base. 6-MMP is in fact converted during acid hydrolysis to 4-amino-5-(methylthio)carboxy imidazole, called 6-MMP derivative. 6-TG, 6-MMP derivative and 8-bromoadenine (8-BA) were resolved on a C18(2) column using gradient elution of 0.05% formic acid and methanol. The three compounds were detected using electrospray ionisation in the positive mode. The ion transitions monitored for 6-TG, 6-MMP derivative and 8-BA were m/z 168 \rightarrow 151, m/z 158 \rightarrow 128 and m/z 216 \rightarrow 199, respectively.

Results: Standard curves were linear up to 2,400 pmol/8x10E8 RBCs for 6-TG and 12,000 pmol/8x10E8 RBCs for 6-MMP derivative ($r > 0.999$). The recovery of both 6-MMP derivative and 8-BA was $> 95\%$ and of 6-TG 80%. For both 6-TG and 6-MMP derivative, bias was $\leq \pm 10\%$, intra- and inter-day coefficients of variation were $< 10\%$, and the limit of quantification was 30 pmol/8x10E8 RBCs. The RBC 6-TGN and 6-MMPN concentration measurement was based on the conversion of 6-TGN and 6-MMPN to 6-TG and 6-MMP derivative. This method is currently used in clinical practice for patients on AZA or 6-MP therapy.

Conclusions: A simple, sensitive and accurate LC-MS/MS method for monitoring of 6-TGN and 6-MMPN has been developed and validated.

7.3

CYP2C19 POOR METABOLISERS: WHO AND WHY?

Nuala Helsby^{1,3}, Kathryn Burns¹, Malcolm Tingle² & Graeme Finlay^{1,3}. ¹Molecular Medicine and Pathology, ²Pharmacology and Clinical Pharmacology, ³Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, University of Auckland, New Zealand

Introduction: The molecular genetics associated with the CYP2C19 poor metaboliser phenotype are used in gene association studies to determine the role of variant *CYP2C19* genotype in therapeutic response. Whilst individuals who are homozygous variant for this gene are invariably poor metabolisers, we have determined that a high proportion of cancer patients, who are not homozygous variant, also display a poor metaboliser phenotype. The mechanism for this is discordance is not known.

Aim: To determine whether *CYP2C19* can be regulated by epigenetic mechanisms.

Methods: HCT-116 cells were treated with DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors for up to 96h. RNA was analysed by qPCR for expression of *CYP2C19*, as well as for some transcription factors reported to be important for *CYP2C19* expression:- *C/EBP-α*, *CAR*, *HNF4a*, *GATA-4*, and *PXR*.

Results: DNMT and HDAC inhibitors increased *CYP2C19* transcription (ΔC_T , $P < 0.001$), with mRNA expression increased up to 26 fold. In addition *GATA-4* mRNA was increased by up to 3600 fold. Five putative CpG islands were identified in the *CYP2C19* sequence. Only one CpG island was upstream of the ATG initiation codon. In contrast, *GATA-4* has at least 15 putative CpG islands and many are associated with the promoter region.

Discussion: *CYP2C19* transcription may be susceptible to epigenetic regulation, either due to altered methylation of CpG islands in the *CYP2C19* gene or methylation changes in the transcription factor *GATA-4*. Studying gene-environment interactions in addition to analysis of SNP of genomic DNA may enhance the relatively meagre translation of pharmacogenetics into clinical practice.

7.4

HIGH TPMT ENZYME ACTIVITY DOES NOT EXPLAIN DRUG RESISTANCE DUE TO PREFERENTIAL 6-METHYLMERCAPTOPYRIMIDINE PRODUCTION IN PATIENTS ON THIOPURINE TREATMENT

Murray L Barclay^{1,2}, Remco Van Egmond^{2,3}, Paul KL Chin², Christopher JJ Mulder³. Departments of Gastroenterology¹ and Clinical Pharmacology², Christchurch Hospital, Christchurch, New Zealand, ³VU University Medical Centre, Amsterdam, Netherlands

Background: Up to 20% of patients on thiopurine therapy fail to achieve adequate drug response. Many of these patients preferentially produce the toxic 6-methylmercaptopyrimidine metabolites (6-MMP) rather than the active 6-thioguanine nucleotides (6-TGN) resulting in a high 6-MMP/6-TGN ratio (>20) and risk hepatotoxicity.

Aim: To determine the prevalence of preferential 6-MMP producers and define the relationships between 6-TGN, 6-MMP and thiopurine methyltransferase (TPMT).

Methods: The database of 6-TGN, 6-MMP and TPMT measurements from patients throughout New Zealand was used to calculate patients' 6-MMP/6-TGN ratios and identify those with high (>20) or normal ratio (≤ 20). TPMT enzyme activity was compared amongst the groups.

Results: Of 1879 patients with TPMT, 6-TGN and 6-MMP results, 349 (19%) had a 6-MMP/6-TGN ratio >20. The mean TPMT enzyme activity was slightly lower for those with a 6-MMP/6-TGN ratio ≤ 20 versus >20, which achieved statistical significance (12.2 vs 13.2; $P < 0.001$). However, the distributions of TPMT enzyme activity were similar, with 97% of TPMT results falling between 5.0 – 17.6 IU/mL for both groups. 17% of those with 6-MMP/6-TGN ratio ≤ 20 were intermediate TPMT metabolizers (TPMT 5.0 – 9.2 IU/mL) versus 7% in those with a ratio >20.

Conclusions: In this patient population with measured 6-MMP/6-TGN ratios, 19% of patients were preferential 6-MMP producers. The results show that high TPMT enzyme activity is not the major reason for preferential 6-MMP production in most patients with a high metabolite ratio. This suggests that there are one or more important alternative mechanisms for preferentially producing 6-MMP.

8. CARNEY SESSION 3 - CHAIR: MURRAY BARCLAY

8.1

ASSOCIATION BETWEEN IMMUNE GENETIC MARKERS AND ALCOHOL DEPENDENCE

Janet K Coller¹, Mark R Hutchinson², Ann K Daly³, Jason M White⁴ & Andrew A Somogyi¹. Discipline of Pharmacology¹, Discipline of Physiology², University of Adelaide, Adelaide, SA; Institute of Cellular Medicine, Newcastle University³, Newcastle upon Tyne, UK; School of Pharmacy and Medical Sciences, University of South Australia⁴, Adelaide, SA

Introduction: Up to 60% of alcohol dependence is heritable. The role of immunogenetics has recently been highlighted via the association between interleukin-1 beta (*IL-1B*) genotypes and alcohol dependence (Liu et al, 2009). However, associations between alcohol dependence and other genetic variants controlling the central immune response are unknown.

Aim: To examine the association between immune genetic variants and alcohol dependence.

Methods: This retrospective case-control study examined the association between the following immune genetic variants (all SNPs) in two alcohol dependent populations (one large, one small) and two healthy control populations: *IL-1B*, *IL-6*, *IL-6R*, *IL-10*, *TNF-a*, *TGF-b*, *TLR2*, *TLR4*, *MD2*, *MYD88*, *BDNF*, *CRP* and *ICE*. Genotypes were determined by Sequenom MassArray Multiplex SNP analysis. Hierarchical cluster analysis was used to determine SNPs that were cumulatively associated with alcohol dependence and was built on data from the large alcohol dependent population (n=567) and a healthy control population (n=200) and subsequently validated in the smaller alcohol dependent (n=93) and healthy control (n=74) populations.

Results: SNPs in the following genes were found to be significantly associated ($P < 0.0001$) with alcohol dependence built with the large population data: *IL-1B*, *IL-2*, *IL-10*, *TNF-a*, *TGF-b*, *TLR2*, *TLR4*, *MD2*, *MYD88*, *BDNF*, *CRP*, *ICE*. A subsequent validation test of this specific cluster in populations in our smaller alcohol dependent (n=93) and healthy control (n=74) populations also revealed a significant association with alcohol dependence ($P < 0.013$).

Discussion: These results demonstrate for the first time that, in addition to *IL-1B* genotype, variability in the loci of multiple immune genes that control the central immune response to alcohol contribute to alcohol dependence.

Liu et al 2009, Pharmacogenetics and Genomics 19:869-76.

8.2

THE BENZBROMARONE STORY - IS CYP2C9 GENOTYPE RELEVANT TO PRESCRIBING?

Rebecca L Roberts^{1,2}, Daniel FB Wright³, Nicola Dalbeth⁴, John Highton⁵, Peter B Jones⁴, Peter J Gow⁶, Lisa K Stamp², Andrew A Harrison⁷ & Tony R Merriman¹. Departments of Biochemistry¹, Pharmacy³, and Medical Sciences⁵, University of Otago, Dunedin, NZ; ²Department of Medicine, University of Otago, Christchurch, NZ; ⁴Department of Medicine, University of Auckland, Auckland, NZ; ⁶Department of Rheumatology, Middlemore Hospital, Auckland, NZ; ⁷Department of Medicine, University of Otago, Wellington, NZ

Introduction: Benzbromarone is an effective uricosuric drug, but was withdrawn from use in many countries after it was linked to serious unexplained hepatotoxicity. The enzyme CYP2C9 plays a critical role in the conversion of benzbromarone to its active metabolite, 6-hydroxybenzbromarone. The clearance of parent drug is significantly lower in individuals who are homozygous for the poor metaboliser (PM) allele *CYP2C9*3*¹. It has been postulated that benzbromarone may be toxic to hepatic mitochondria², suggesting that PMs may be at a heightened risk of benzbromarone-induced hepatotoxicity.

Aim: To determine the frequency of *CYP2C9*3* and another PM allele, *CYP2C9*2*, in New Zealand Caucasian and Polynesian gout cohorts.

Methods: 852 Caucasians (537 controls, 315 gout patients) and 1072 Maori and Pacific Island (Polynesian) people (620 controls, 452 gout patients) were genotyped for *CYP2C9*2* and *CYP2C9*3* using predesigned TaqMan SNP assays.

Results: Frequency of *CYP2C9* PM alleles was significantly higher in Caucasians compared to Polynesians (*CYP2C9*2*: 13.5% versus 3.1%; *CYP2C9*3*: 5.5% versus 1.6%, $p < 1.2E-11$). Within Polynesians *CYP2C9* PM alleles were rarer in Western Polynesians (Samoa, Tonga) than Eastern Polynesians (NZ and Cook Island Maori; *CYP2C9*2*: 0.6% versus 2.5%; *CYP2C9*3*: 0.4% versus 2.0%; $p < 0.03$). The frequency of *CYP2C9* PM alleles did not differ between population-matched controls and gout patients ($p > 0.107$). None of the Polynesian gout patients had a *CYP2C9* PM genotype, whereas 5.4% of Caucasian patients carried two PM alleles.

Discussion: If *CYP2C9* PM genotype is found to increase risk of benzbromarone-induced hepatotoxicity, prospective genotyping of Caucasian gout patients may be warranted. The absence of genotypic *CYP2C9* PMs in Polynesians suggests that the *CYP2C9* polymorphism may be of limited clinical relevance to this group.

8.3

PERSONALISED THIENOPYRIDINE THERAPY: THE COST EFFECTIVENESS OF GENETIC TESTING FOR CYP2C19 VARIANTS TO GUIDE TREATMENT IN PATIENTS WITH ACUTE CORONARY SYNDROMES

¹Laura Panattoni, ¹Braden Te Ao, ^{1,2}Paul Brown & ¹Patrick Gladding¹. University of Auckland, Auckland, NZ,
²University of North Carolina, Chapel Hill

Introduction: Previous studies suggest prasugrel may be cost effective when compared to clopidogrel for treating acute coronary syndrome patients (ACS). Recent research has shown that the reduced function allele CYP2C19*2 (*2 allele) is associated with an increased risk of adverse events for ACS patients taking clopidogrel and a decreased risk for patients taking prasugrel. The purpose of this paper is to test whether using generic clopidogrel for all patients is cost effective compared to a) prasugrel for *2 allele patients and clopidogrel for non *2 patients and b) to prasugrel only for New Zealand.

Methods: Effectiveness of clopidogrel and prasugrel from published TRITON-TIMI 38 (n=13,608) clinical trials was combined with rates of *2 occurrence in Maori, Pacific Islanders, Asian and NZ European and national hospital records on rates and costs of hospitalisations 15 months post ACS for stroke, MI, bleeding, stent thrombosis and cardiovascular death. Decision tree modelling and Monte Carlo simulations examined the robustness of the results.

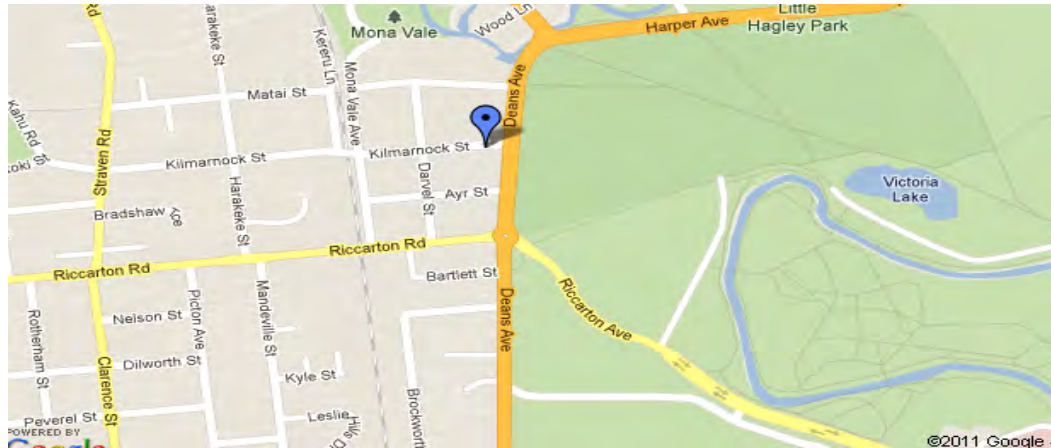
Results: Rates of the *2 allele differ significantly between NZ European (15%), Maori (24%), Asian (29%) and Pacific People (45%). Analysis of hospital records suggest that rates of MI, stroke, bleeding, stent thrombosis and cardiovascular death were much higher in the general New Zealand population than in the clinical trial population. The cost effectiveness analysis suggests that use of a genetic test to guide combined use of clopidogrel and prasugrel was cost effective for most age and ethnic groups, but particularly for Maori males (NZ\$3184/QALY), Maori females (NZ\$3687/QALY), Pacific men (NZ\$4617/QALY) and Pacific women (NZ\$7605/QALY). Prasugrel is more costly and less effective when used in isolation compared to genetically guided thienopyridine treatment.

Discussion: The results here suggest that the use of a genetic test to guide treatment decisions for ACS patients is cost effective, especially for Maori and Pacific peoples, and that prasugrel alone is not cost effective in New Zealand.

GENERAL INFORMATION

HOSTS Clinical Pharmacology, Christchurch Hospital, 2 Riccarton Road, Christchurch
 Department of Medicine, University of Otago, 2 Riccarton Road, Christchurch
 T: +64 3 364 1055
 F: +64 3 364 1003

VENUE *The Camelot Room, The Chateau on the Park*
 189 Deans Avenue, Riccarton, Christchurch



REGISTRATIONS	Sunday, 28 August	6.00 - 7.30pm	Camelot Room
SOCIAL FUNCTIONS	Monday, 29 August	7.00pm	Camelot Room
REFRESHMENTS	Breaks & Lunch		Camelot Room
AGM	Monday, 29 August	4.30pm	Camelot Room

ASCEPT NZ AND CARNEY CONFERENCE ORGANISING COMMITTEE 2011

Chair	Evan Begg and Martin Kennedy
Deputy Chair	Murray Barclay
Treasurer	Paul Chin
Secretary	Berit Jensen
Admin Support	Jane Vella-Brincat, Julie Humphries

ACKNOWLEDGEMENTS

We would like to thank the following organisations for their support of this meeting:



