Christchurch Medical Research Society

AGM and Scientific Meeting

Wednesday 28 April 2004

5 pm - 7.45 pm in the Rolleston Lecture Theatre

5:00 ANNUAL GENERAL MEETING

5:15 ORAL PRESENTATIONS

5:15 **Hyperlipidemia and the liver sieve**
Victoria C Cogger, Sarah N Hilmer, Robin Fraser, David G Le Couteur

5:30 **3D Models of Blood Flow in the Cerebral-Vasculature**
Stephen Moore, Geoffrey Chase, John Fink, Tim David

5:45 **A new Ras inhibitor for advanced breast and prostate cancer**
Mandy Lynch, Richard Santen, Rob McPherson

6:00 REFRESHMENTS

There will be no charge for refreshments as this is covered by the $10 subscription which will be gladly accepted at the meeting!!

6:30 ORAL PRESENTATIONS CONT.

6:30 **Cytomegalovirus, Epstein-Barr virus and risk of breast cancer before age 40 years: a case-control study**
Ann Richardson, Brian Cox, Margaret McCredie, Gillian Dite, Jiun-Horng Chang, Dorota Gertig, Melissa Southey, Graham Giles, John Hopper

6:45 **Role of glutathione in the resistance of Bcl-2 positive cells to Fas-mediated apoptosis**
Susan Thomson, Juliet Pullar, Mark Hampton

7:00 **Comparison of Faecal Calprotectin and Clinical Disease Indices for Assessing Inflammation in Crohn's Disease**
Richard Gearry, Murray Barclay, Michael Burt, Bruce Chapman, Judith Collett, Peter George

7:15 **Factors determining efficiency of Der f l and Fel d l removal from carpet**
Simon Causer, Caroline Shorter, Roger Lewis

7:30 **Effect of Hyperglycemia on Mortality in the Christchurch Intensive Care Unit**
Carmen Doran, Maxim Bloomfield, Dominic Lee, Geoffrey Shaw, Geoffrey Chase, Jessica Lin, Thomas Lotz

Christchurch Medical Research Society

34th Annual General Meeting, 28 April 2004

Agenda

1. Apologies: John Evans, Martin Kennedy, Jinny Willis, Russell Scott
2. Minutes of the 33rd AGM held on 16 April 2003 (attached)
3. Chairperson's report – Geoff Shaw (attached)
4. Financial statement – Michael MacAskill (attached)
5. Election of Executive

The following members of the Committee offer themselves for re-election.

Chairperson	Geoff Shaw – Intensive Care
Secretary/Treasurer	Michael MacAskill – Medicine
Committee	Dru Mason – Zoology, Canterbury University
	Elisabeth Wells – Public Health
	John Fink – Neurology
	Chris Pemberton – Endolab
	Darin O'Keeffe – Medical Physics

New member: Professor Tim David, Mechanical Engineering, Canterbury University.

Thanks to retiring Executive members:
Karl Sluis, David McGregor

6. General business

Minutes of the 33rd Annual General Meeting, 16 April 2003, held in the Beaven Theatre, Christchurch School of Medicine

Opening: The meeting was opened at 5:03 p.m.

Present: Dr Geoff Shaw (in the chair) and 33 members.

Apologies: Ian Town, Ross Bowie, John Evans, Margaret Currie, Karl Sluis

Chairperson's report: Dr Geoff Shaw presented the chairperson's report.

Financial statement: The financial statement to 31 March 2003 was presented by the Secretary/Treasurer (Dr Michael MacAskill) and accepted by the meeting. Subscriptions were maintained at $10.00 for 2003/2004.

Election of officers: Darin O'Keeffe was elected to fill the vacancy on the Committee. The Committee for 2003/2004 will be as follows:

Chairperson	Geoff Shaw – Intensive Care
Secretary/Treasurer	Michael MacAskill – Medicine
Committee	Dru Mason – Zoology, Canterbury University
	David McGregor – Nephrology
	Robyn Niven – Research Office
	Karl Sluis – Biorad Ltd
	Elisabeth Wells – Public Health
	John Fink – Neurology
	Chris Pemberton – Endolab
	Darin O'Keeffe – Medical Physics

General business.

Thanks to retiring Executive members

Dr Richard Tremewan retired from the Executive Committee and was thanked for his efforts over the years.

Banking matters

The Treasurer noted that the Society's bank, WestpacTrust, no longer paid the small amount of interest on the balance of our non-profit check

account. It was resolved that the Committee should investigate alternative banking arrangements so as to increase the Society's income from its cash reserve (moved M. MacAskill, seconded E. Wells, carried U.)

Discussion

1. Richard Tremewan suggested that we should publicise our meeting via the Royal Society's newsletter.
2. Christine Winterbourne suggested that the Committee should extend an invitation to a senior researcher to give an extended (say 30 minute) talk at CMRS meetings.

Closure

There being no further business, the AGM closed at 5:13 pm.

CMRS Chair's 2003/04 Annual Report

This is the 34th AGM of the society and the CMRS continues to go from strength. The 2003 presentations at the CMRS have been of a very high standard and the numbers attending these sessions continue to increase. In addition the number of papers put forward frequently exceeds the time available. It was partly for this reason the CMRS and the Canterbury Medical Research Foundation are supporting the inaugural 'Canterbury Health Science Research Conference' to be held in conjunction with the CMRF Open Day, 5-6 September 2004. It is anticipated this conference will showcase the world-class research being carried out in Canterbury and will provide a platform to bring researchers, their sponsors and the public together. A healthy debate amongst researchers and invited politicians on the future direction of medical research is also planned. Your support for this meeting is vital. Details can be found at:

http://www.cmrf.org.nz/index.php?page=conference

For the last two years 3M have sponsored a prize of $300 for the best oral presentation. The Society would like to thank the continued support of 3M and congratulate last year's recipients:

1st equal: Dr Gabi Dachs and Anna Pilbrow
Certificates of merit: Dr Richard Gearry and Annna Mitchell.

My thanks to Robyn Niven and Michael MacAskill for their unfailing support in pulling the scientific sessions together.

Finally a huge vote of thanks to the presenters, their supporters, and members who continue to infuse the Society with their knowledge, wits, and skills. This is what creates the sense of community amongst researchers.

Geoffrey M Shaw
Chair
26th April 2002

Christchurch Medical Research Society

Annual Financial Statement to 31 March 2004

Balance brought forward (as at 31.03.03)		$3 296.15
Income	**2002/2003**	**2003/2004**
Subscriptions	760.00	500.00
Sponsorship: 3M	220.00	300.00
Total	980.00	800.00
Expenditure		
Catering	280.00	320.00
Drinks	0.00	149.36
Prizes	400.00	600.00
Total	680.00	1069.36
Income over expenditure	**300.00**	**(269.36)**
Current balance as at 31.03.04		**$ 3 026.79**

Notes:
Unexpected extra expenditure due to awarding two first prizes. CMRF has undertaken to donate $200 to cover this. Will appear in next year's accounts.

Recommendations:

1 – That the report be received

2 – That the annual subscription remain at $10 for 2004/2005

3 – That the Executive open a Westpac Bonus Saver Account and deposit into it a portion of the Society's funds.

Michael R. MacAskill
Secretary/Treasurer

Hyperlipidemia and the liver sieve

Victoria C Cogger[1], Sarah N Hilmer[1], Robin Fraser[2], David G Le Couteur[1]

[1] Centre for Education and Research on Ageing and ANZAC Research Institute, University of Sydney, Australia

[2] Department of Pathology, University of Otago, Christchurch, New Zealand

The hepatic sinusoidal endothelium (liver sieve) is perforated with numerous fenestrae each of about 100nm in diameter. The fenestrations allow passage of substrates such as chylomicron remnants but exclude larger particles such as chylomicrons. Alterations in the liver sieve are known to affect hepatic lipoprotein uptake.

A single intraperitoneal (i.p.) injection of Poloxamer 407 (P-407) (0.5-1.0mg/kg) induces dramatic hyperlipidemia within 24 hours in rodents. The precise mechanism for these changes is not understood. One possible explanation is that P-407 alters the structure of the liver sieve, impairing the transfer of lipoproteins across the liver sieve. We report the affects of a single i.p. dose of P-407 on the plasma lipids and on the liver sieve porosity.

Ten week old, male BL57 mice were injected with either P-407 (1mg/kg, i.p., n=5) or volume matched normal saline (i.p., n=5). After 24 hours the mice were anaesthetised, blood samples were taken for triglyceride and cholesterol analysis, and the livers were perfusion fixed via the portal vein (3% Glutaraldehyde/2.5% Paraformaldehyde). Livers were then prepared for scanning electron microscopy.

At 24 hrs total triglycerides and cholesterol were significantly elevated in the P-407 treated animals when compared with controls (triglycerides: 15.4 ± 4.7 v 2.1 ± 4.3, $P < 0.01$, and cholesterol: 6.9 ± 1.4 v 2.6 ± 0.1, $P < 0.01$). Scanning electron microscopy of the liver sieve showed dramatic loss of endothelial fenestrations in the P-407 treated animals when compared with the controls.

Administration of P-407 is associated with structural changes to the liver sieve that may impair hepatic uptake of lipoproteins with resulting hyperlipidemia.

3D Models of Blood Flow in the Cerebral-Vasculature

S. Moore[1], J. G. Chase[1], J. Fink[2] and T. David[1]

[1] Centre for Bioengineering, University of Canterbury

[2] Christchurch School of Medicine and Health Sciences, University of Otago

The Circle of Willis (CoW) is a ring-like arterial structure located in the base of the brain, responsible for the distribution of oxygenated blood throughout the cerebral mass. Among the general population, approximately 50% have a complete CoW, where among a multitude of possible anatomical variations, vessels absent from the CoW are common. Certain conditions such as a build up of atherosclerotic plaque on the arterial wall can result in ischaemic damage and stroke-like symptoms. A 3D computer model has been developed based on the results of a Magnetic Resonance Angiogram of a patient's cerebral vasculature and a numerical algorithm to simulate the body's autoregulation mechanism. The intention of the present study was to simulate different pathological states, including different vessels missing from the circle and varying degrees of stenosis in an afferent Internal Carotid Artery.

Results show that the CoW is remarkably resilient to a stenosis in one of the Internal Carotid arteries. Because of their small diameters the communicating arteries act almost as barriers between pieces of the circle and it is not until there is sufficient asymmetric afferent blood pressure that any significant amounts of blood will flow through them. The contralateral Posterior Communicating artery plays virtually no role in the re-distribution of blood to the starved ipsilateral side of the CoW. Peripheral resistances decrease to increase blood flow through the communicating arteries which also increases afferent flow, even when the Internal Carotid artery is subjected to a pressure drop. This indicates that 'pressure' is the more physiologically correct boundary condition as opposed to the specification of an inlet 'velocity profile'.

A new Ras inhibitor for advanced breast and prostate cancer

Mandy Lynch[1], Richard Santen[1] and Rob McPherson[1, 2]

[1]Division of Endocrinology and NCI Cancer Center, University of Virginia, Charlottesville, Virginia, USA

[2] Current address: Canesis Networks Ltd., Lincoln, New Zealand. rob.mcpherson@canesis.com

Resistance to hormonal therapy of advanced breast and prostate cancer is a serious clinical problem. Altered regulation of signal transduction pathways are a hallmark of resistance to hormonal therapy in vivo and in vitro. The 21 kDa Ras GTP binding protein is a key mediator of these activated signal transduction pathways. Treatments targeting Ras have been extensively researched as cancer therapies but are yet to find clinical applications. The new Ras inhibitor farnesylthiosalicylate (FTS) has a very good therapeutic index in rodent models and is effective against other cancers in vitro that have upregulated Ras. We hypothesised that FTS would be an effective treatment for breast and prostate cancers that have developed resistance to hormonal therapy.

We confirmed that Ras was upregulated in cellular models of advanced breast and prostate cancer. Inhibition of signal transduction pathways downstream of Ras arrested growth of these cells. The anti-Ras compound FTS disrupted signal transduction in cellular models of advanced breast and prostate cancer significantly. Accompanying this disruption was massive cellular apoptosis and reduced proliferation. FTS also induced degradation of important cellular proteins, indicating it either increased turnover or reduced transcription/translation. A soluble complex of FTS, suitable for human dosage in vivo, was just as effective as FTS alone. FTS was also additive with Doxorubicin against a cellular model of advanced breast cancer.

FTS is effective against in vitro models of breast and prostate cancers that are resistant to hormonal therapy. FTS is entering preclinical trials for human cancer.

Cytomegalovirus, Epstein-Barr virus and risk of breast cancer before age 40 years: a case-control study

Ann K Richardson[1], Brian Cox[2], Margaret RE McCredie[2], Gillian S Dite[3], Jiun-Horng Chang[3], Dorota M Gertig[3], Melissa C Southey[4], Graham G Giles[5], John L Hopper[3]

[1]Department of Public Health and General Practice, Christchurch School of Medicine and Health Sciences, University of Otago, PO Box 4345 Christchurch, New Zealand

[2]Department of Preventive and Social Medicine, Dunedin School of Medicine, University of Otago, PO Box 913 Dunedin, New Zealand

[3]Centre for Genetic Epidemiology, University of Melbourne, Victoria 3053, Australia

[4]Department of Pathology, University of Melbourne, Victoria 3010, Australia

[5]Cancer Epidemiology Centre, The Cancer Council Victoria, Melbourne, Victoria 3053, Australia

The aim of this paper was to investigate whether there is an association between cytomegalovirus (CMV) and Epstein-Barr virus (EBV) IgG levels and risk of breast cancer before age 40 years. CMV and EBV IgG levels were measured in stored plasma from 208 women with breast cancer and 169 controls who participated in the Australian Breast Cancer Family Study (ABCFS), a population-based case-control study. CMV and EBV IgG values were measured in units of optical density (OD). Cases and controls did not differ in seropositivity for CMV (59% and 57% respectively; p = 0.8) or EBV (97% and 96% respectively; p = 0.7). In seropositive women, mean IgG values were higher in cases than controls for CMV (1.20 versus 0.98 OD, p = 0.005) but not for EBV (2.65 versus 2.57 OD, p = 0.5). The adjusted odds ratios per OD unit were 1.46 (95% CI 1.06 to 2.03) for CMV IgG and 1.11 (0.93 to 1.33) for EBV IgG. The higher mean CMV IgG levels found in women with breast cancer could be the result of a more recent infection with CMV, and may mean that late exposure to CMV is a risk factor for breast cancer.

Role of glutathione in the resistance of Bcl-2 positive cells to Fas-mediated apoptosis

Susan J. Thomson, Juliet M. Pullar, Mark B. Hampton

Free Radical Research Group, Department of Pathology, Christchurch School of Medicine & Health Sciences

Increased expression of the *bcl-2* oncogene has been linked to carcinogenesis and drug-resistance in a range of cancers. Bcl-2 functions by blocking apoptosis, but its exact mechanism of action is unclear. Overexpression of Bcl-2 is reported to elevate levels of the cysteine-containing tripeptide glutathione (GSH), and this has been proposed to interfere with apoptosis. Our aim was to test the link between Bcl-2 levels and intracellular GSH, and determine whether elevated GSH provides a survival advantage to cells. We generated a range of stable Jurkat lymphoma cells over expressing Bcl-2 at levels ranging from 2 to 50 times that of parent cells. Bcl-2 expressing clones exhibited a 60% average increase in GSH. We observed a strong negative correlation between Bcl-2 protein levels and caspase activation following Fas induced apoptosis (Pearson product moment correlation $r = -0.625$, $p=0.01$). However, there was no correlation between Bcl-2 and GSH, or between GSH and resistance to apoptosis. Also, depletion of GSH with buthionine sulfoximine had no effect on the sensitivity of the cells to Fas-mediated apoptosis. However, phenethyl isothiocyanate (PEITC) was able to sensitize the Bcl-2 positive clones. PEITC lowers GSH and can directly modify cellular thiol proteins. While we conclude that Bcl-2 elevates GSH and promotes an intracellular reducing environment, GSH itself does not contribute to the anti-apoptotic properties of Bcl-2. We propose that PEITC bypasses the action of Bcl-2 by directly modifying specific thiol proteins. Identification of these proteins may provide novel targets in the search for novel treatments of drug-resistant cancers.

Comparison of Faecal Calprotectin and Clinical Disease Indices for Assessing Inflammation in Crohn's Disease

Richard B. Gearry[1, 3], Murray L. Barclay[1, 2, 3], Michael J Burt,[1, 3] Bruce A Chapman,[1] Judith A Collett,[1] Peter George.[4]

Departments of Gastroenterology[1] and Pharmacology[2] Christchurch Hospital, Department of Medicine, Christchurch School of Medicine and Health Sciences[3] and Canterbury Health Laboratories,[4] Christchurch, New Zealand.

Faecal calprotectin (FC) is a sensitive marker of intestinal inflammation that correlates well with invasive methods of inflammation assessment in Crohn's disease (CD) patients. We aimed to compare FC with conventional laboratory measurements of inflammation and clinical indices of disease activity - Crohn's Disease Activity Index (CDAI) and the short Inflammatory Bowel Disease Quality of Life score (sIBDQ).

This study had ethical approval and patients gave written informed consent. Patients with CD were recruited for the study from outpatient clinics or inpatient wards. Stool specimens for FC, blood for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and highly sensitive CRP (hs-CRP) was collected. The patients completed symptom cards (CDAI) and questionnaires (sIBDQ). Laboratory work was carried out by Canterbury Health Laboratories and clinical assessments by the principle investigator. The results were correlated using 2-tailed Pearson correlation coefficients.

Thirty-seven of 46 (80%) recruited patients with Crohn's completed the study (28 (76%) female, mean age 36y). The FC range was 2 to 12282 mg/L. Correlation coefficients were as follows: CDAI v sIBDQoL r=0.869**, Log(FC) v CDAI r=0.328*, Log(FC) v sIBDQ r=0.235, Log(FC) v Log (ESR) r=0.608**, Log(FC) v Log(CRP) r=0.560**, Log(FC) v Log(hs-CRP) r=0.555**. (**$p<0.001$, *$p<0.05$).

The simpler sIBDQoL questionnaire correlates extremely well with CDAI. HsCRP was no better than CRP in determining CD activity. FC correlates modestly with conventional laboratory measures of inflammation. FC may be a better indicator of IBD inflammatory activity than symptom scores and standard laboratory measures, particularly at lower levels of inflammation.

Factors determining efficiency of Der f I and Fel d I removal from carpet

Simon M. Causer[1], Caroline L. Shorter[1], and Roger D. Lewis[2]

[1] Canesis Network, Christchurch, New Zealand

[2] Saint Louis University School of Public Health, St. Louis, Missouri, USA

Allergens derived from house-dust mites and pets, exposure to which is a risk factor for the development of sensitization and asthma, are known to accumulate in carpets. Regular vacuuming has been suggested as a useful technique for reducing exposure to such material, however, the efficiency of such processes is poorly understood. We sought to examine how wear and cleaning method affected house-dust mite (Der f I) and cat allergen (Fel d I) removal from carpet.

A selection of wool carpets (cut- and loop-pile of 7 mm or 9 mm pile height, 6 replicates each) were loaded with fine sieved house dust containing known amounts of Fel d I or Der f I, artificially worn, and subjected to either dry or wet vacuuming. The Der f I and Fel d I content of carpet cores was determined using double-monoclonal antibody ELISA before and after wear, and after cleaning.

Both dry and wet vacuuming procedures were effective at removing Der f I (>61%) and Fel d I (>74%) from unworn carpets, but this efficiency reduced to <30% for Der f I and to <39% for Fel d I once worn. Cleaning method and carpet style (cut vs loop) had no significant impact on ease of allergen removal ($P>0.05$), and pile height had only a minor influence.

We conclude that the degree of wear is one of the most important factors to consider when advising allergen sensitive patients of techniques to minimise allergen levels in carpet.

Effect of Hyperglycemia on Mortality in the Christchurch Intensive Care Unit

Carmen V. Doran[1], Maxim Bloomfield[2], Dominic Lee[3], Geoffrey M. Shaw[4], J. Geoffrey Chase[1], Jessica Lin[1], Thomas Lotz[1]

[1] Biomedical Engineering Research Group, Department of Mechanical Engineering, University of Canterbury

[2] Christchurch School of Medicine and Health Science, University of Otago

[3] Department of Mathematics and Statistics, University of Canterbury

[4] Department of Intensive Care, Christchurch Hospital

Critically ill patients often experience stress-induced hyperglycemia. Increased counter-regulatory hormone secretion leads to increased endogenously produced glucose and hepatic gluconeogenesis, and reduces insulin sensitivity. High dextrose nutritional support regimes compound the counter-regulatory response.

During 2003, 1164 patients were admitted to the Christchurch Hospital ICU. A retrospective data audit analysed relationships between plasma glucose values, insulin infusions, age, sex, APACHE II score, length of stay, ICU mortality and primary diagnosis for the 201 patients with a stay of greater than 72 hours.

The number of measurements per day, maximum, range and trapezoidal mean of blood glucose were higher in non-survivors ($P = 0.03$, $P = 0.001$, $P = 0.003$ and $P = 0.014$, respectively). Insulin infusion averages and proportion of stay were negatively correlated with mortality ($P = 0.01$ and $P = 0.02$, respectively). The general trend was that survival decreased with increasing maximum blood glucose, range of blood glucose and trapezoidal mean of blood glucose, suggesting that these three parameters have a negative effect on the survival of an ICU patient, independent of APACHE II scores.

Finally, the error in estimating the mean blood glucose for a patient over an interval of time increased with sampling time. Standard practice of four hourly measurements may not be appropriate for tightly controlling glucose levels, further reiterating the need for glucose sensing technology and automated insulin infusion in critical care environments.