Christchurch Medical Research Society

AGM and Scientific Meeting

Wednesday 24 April 2002

5 pm - 7.30 pm in the Rolleston Lecture Theatre

5.00 ANNUAL GENERAL MEETING

5.15 ORAL PRESENTATIONS

5.15 VEGF-D expression is associated with lymph node status in primary human breast carcinomas and regulated by estrogen in breast carcinoma cell lines
Margaret J Currie*, Sarah P Gunningham, Vickie Hanrahan, Helen R Morrin, Prudence A E Scott, Bridget A Robinson, Stephen B Fox

5.30 Alcohol use and misuse among older people in the community: hidden problem
Nadim Khan*, Peter Davis, Tim J Wilkinson, Douglas J Sellman, Patrick Graham

5.45 REFRESHMENTS

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

6.10 POSTER PRESENTATIONS

Gene expression of VEFG-A, VEGF-B, VEGF-C and their tyrosine kinase receptors in human colorectal cancer
Vickie Hanrahan, Margaret J Currie, Sarah P Gunningham, Helen R Morrin, Prudence AE Scott, Bridget A Robinson and Stephen B Fox

Patterns of alcohol use and misuse among elderly rest-home residents in Christchurch, New Zealand
Nadim Khan, Tim J Wilkinson, Douglas J Sellman, Patrick Graham

6.15 ORAL PRESENTATIONS CONT.

6.15 Pressure controlled ventilation compared with volume controlled ventilation in anaesthetised adult patients
R A French*

6.30 Oxidation of β_2-agonists by peroxidases and its relevance to asthma control
Stephen J Hoskin*, Anthony J Kettle

6.45 Patient agitation and heart rate variability
Z H Lam*, S Hunt, J Geoffrey Chase, Dr Geoff Shaw

7.00 Sialic acid content of fibrinogen in pregnant women and in individuals on fibrate therapy
G Maghzal*, S Brennan, P George

7.15 C-type natriuretic peptide (CNP) and amino terminal pro C-type natriuretic peptide (NT-proCNP) in a sheep model of mild sepsis
Timothy C R Prickett*, Timothy G Yandle, Graham K Barrell, Martin Wellby, M Gary Nicholls, Eric A Espiner, A Mark Richards

* Contestants for the CMRS Young Researcher Prize

Christchurch Medical Research Society

32nd Annual General Meeting, 24 April 2002

Agenda

1. Apologies: Don Beaven, Jack Heinemann, David McGregor, Ross Bowie
2. Minutes of the 31st AGM held on 18 April 2001 (attached)
3. Chairperson's report – Richard Tremewan (attached)
4. Financial statement – Michael MacAskill (attached)
5. Election of Executive

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
	Richard Tremewan – Medical Physics
	Elisabeth Wells – Public Health
	John Fink – Neurology*
	Chris Pemberton – Endolab**

* Nominated Tim Anderson, seconded Michael MacAskill

** Nominated Chris Charles, seconded Michael MacAskill

6. General business

Thanks to retiring Executive members:
Barbara Peddie, Chris Charles, Tim Anderson, John Elliott, Steven Gieseg.

Minutes of the 31st Annual General Meeting, 18 April 2001, held in the Rolleston Theatre, Christchurch School of Medicine at 5.15 pm.

Present: Dr Richard Tremewan (in the chair) and 20 members

Apologies. Ian Town, Karl Sluis, Nancy Gould, Chris Charles, Mike Ardagh (Accepted)

Minutes. The minutes of the 30th AGM held on April 12, 2000 were ratified.

Chairperson's report. Dr Richard Tremewan presented the chairperson's report which was received with acclaim. There was some discussion about the annual subscription ($50) which the CMRS pays to the CMRF. The question of using income for advertising was raised.

Financial statement. The financial statement to 31 March 2001 was presented by the Secretary/Treasurer (Dr Barbara Peddie) and accepted by the meeting. Subscriptions were maintained at $10.00 for 2001/2002.

Election of officers. The Committee for 2001/2002 will be as follows:

Chairperson:	Dr Richard Tremewan -Medical Physics
Secretary	Dr Barbara Peddie - Nephrology
Treasurer	Dr Steven Geisig -Zoology, Canterbury University
Committee	Dr Tim Anderson - Neurology
	Dr Chris Charles - Medicine
	Dr Dru Mason - Zoology, Canterbury University
	Dr Karl Sluis - Biorad Ltd
	Dr Elisabeth Wells - Public Health
	Dr John Elliott - Cardiology
	Dr Geoff Shaw - Intensive Care
	Dr Michael MaAskill - Medicine
	Dr David McGregor, Nephrology.

General business.
There being no further business, the AGM closed at 5.20 pm.

CMRS Chair's 2001/02 Annual Report

This is the 32nd AGM of the Society. I am pleased to report that after 31 years the CMRS remains in good health and well supported by the health research community.

Sponsorship from 3M has allowed us to continue the "young" researcher prize which attracts consistently good presentations. My thanks to the judges Robin Fraser, Martin Kennedy, Dru Mason and Geoff Shaw.

In 1979 Robin Carrell, the then Chair of the CMRS, wrote of the continuing need for a broad interest society to provide an overall forum for the presentation of medical research. This comment arose because of some loss of momentum caused by the formation of specialist societies. Robin also spoke of how the society was helping develop a sense of community amongst researchers. More than 20 years later the concept of a broad interest society and the development of a sense of community remain pertinent and I hope they will continue to invigorate the Society.

My thanks to the Research Office and especially Robyn Niven for her support. Thank you also to the members of the CMRS executive who have so enthusiastically supported the Society. Particular acknowledgment is due to retiring members Barbara Peddie, Chris Charles, Steven Gieseg and Tim Anderson. Barbara, was Secretary/Treasurer for many years and has kept the Society in splendid order. Chris Charles was secretary/treasurer from 1993-96, has been the victualler and often managed the audio-visuals for more years than any other executive member can remember.

And of course thank you to all members of the Society for helping to create a sense of community amongst health researchers.

Richard Tremewan
Chair
24 April 2002

Christchurch Medical Research Society

Annual Financial Statement to 31 March 2002

Balance brought forward (as at 31.03.01) $2909.99

Income	**2001**	**2002**
Subscriptions	805.00	425.00
Sponsorship: 3M	300.00	300.00
Interest	44.16	34.05
PAYE Refund	6.13	6.63
Total	1155.29	765.68

Expenditure		
Petty cash	0.00	0.00
Photocopying	63.88	0.00
Meals (including drinks)	447.80	344.65
PAYE	8.61	6.63
Cheque Book	0.95	0.00
Membership, CMRF	50.00	0.00
Prizes	400.00	600.00
Total	971.24	480.99

Income over expenditure	**$184.05**	**($185.60)**

Current balance as at 31.03.01 $2724.99

Recommendations:
1 - that the report be received
2 - that the annual subscription remain at $10 for 2002-2003

Michael R. MacAskill (Secretary/Treasurer)

VEGF-D expression is associated with lymph node status in primary human breast carcinomas and regulated by estrogen in breast carcinoma cell lines.

Margaret J. Currie[1], Sarah P. Gunningham[1], Vickie Hanrahan[1], Helen R. Morrin[1], Prudence A.E Scott[2], Bridget A. Robinson[2], Stephen B. Fox[3].

[1]Angiogenesis Research Group, University of Otago, Christchurch School of Medicine, P.O.Box 4345, Christchurch, New Zealand.

[2]Oncology Department, Canterbury Health, Christchurch Hospital, Christchurch, New Zealand.

[3]Nuffield Department Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom.

Angiogenesis, the formation of new blood vessels from existing vasculature, is essential for tumour growth and metastasis. Vascular Endothelial Growth Factor-D (VEGF-D), a recently identified VEGF family member, is a potent angiogenic factor *in vivo* and stimulates endothelial cell proliferation and migration *in vitro*. Although overexpression of VEGF-D has been shown to promote tumour cell metastases in animal studies, its contribution to tumour neovascularization and metastasis in human breast tumours is unknown. We therefore measured the level of VEGF-D mRNA by relative RT-PCR in 10 normal and 53 invasive breast cancers and correlated their level of expression with standard clinicopathological parameters. Tumours expressed more VEGF-D than normal breast tissue, and VEGF-D gene expression was significantly associated with tumour size ($p=0.05$), nodal status ($p=0.008$), and the number of involved nodes ($p=0.0053$), but not with patient age, estrogen receptor (ER)($p=0.209$), progesterone receptor ($p=0.193$), tumour histology ($p=0.136$), grade ($p=0.796$), vascular invasion ($p=0.645$), or expression of VEGF-D receptors KDR ($p=0.935$) and flt-4 ($p=0.589$). Since VEGF family members have been shown to be estrogen regulated we assessed the effect of 17β-estradiol on VEGF-D mRNA expression in a panel of ER positive (MCF-7, T47D) and ER negative (MDA231, MDA453, MDA435, MDA468, BT20, SkBR3) breast carcinoma cell lines. We observed that physiological amounts of estrogen (1nM) significantly upregulated VEGF-D in T47D cells at 18 hours ($p<0.01$). These data suggest that VEGF-D expression may provide a surrogate marker of nodal metastasis that may be used to stratify patients for adjuvant chemotherapy, and give additional support for targeting VEGF receptors as an anti-cancer therapy.

Alcohol use and misuse among older people in the community: hidden problem

Nadim Khan[1], Peter Davis[3], Tim J Wilkinson[1], Douglas J Sellman[2], Patrick Graham[3]

[1]Health Care of the Elderly, Department of Medicine, [2]Department of Psychological Medicine and [3]Department of Public Health and General Practice, Christchurch School of Medicine and Health Sciences, University of Otago

To determine patterns of alcohol use and misuse among community-dwelling people aged 65 years and over in Christchurch and to assess how often this comes to medical attention, a cross-sectional survey of alcohol use and misuse was conducted followed by a self-administered postal survey among non- respondents. GPs of the respondents completed a self-administered questionnaire on patients' alcohol use and misuse.

The response rate was 58% (141/243). The prevalence of hazardous alcohol consumption in the past 12 months (AUDIT cut-off score 8 or more) was 9.9% (95% CI = 4.9-14.9) and the prevalence of lifetime alcohol dependence using DSM-IV diagnostic criteria was 24.8% (95% CI = 17.6-32.0). Men were more likely than women to report lifetime dependency and current hazardous patterns. The response rate among GPs was 77.7% (108/139).

None of the GPs who responded identified or diagnosed any alcohol problems in the past 12 months among this group and reported a history of alcohol problems in only 4 (4.0%) patients. Those with current hazardous patterns of alcohol use were twice as likely to be admitted to hospital (RR=2.4; 95% CI 1.2-5.1) but significantly less likely to visit their GPs in the previous 12 months (RR=0.55; 95% CI 0.7-1.1).

A significant proportion of community-dwelling elderly people reported patterns of alcohol consumption that put them at risk of future damage to physical or mental health. Hazardous drinkers were less likely to visit their GPs and only in a few cases, were GPs aware of such potential problems.

Gene expression of VEGF-A, VEGF-B, VEGF-C and their tyrosine kinase receptors in human colorectal cancer.

Vickie Hanrahan[1], Margaret J. Currie[1], Sarah P. Gunningham[1], Helen R. Morrin[1], Prudence A.E. Scott[2], Bridget A. Robinson[2] and Stephen B. Fox[3].

[1]Angiogenesis Research Group, University of Otago, Christchurch School of Medicine, P.O.Box 4345, Christchurch, New Zealand.

[2]Oncology Services, Christchurch Hospital, Christchurch, New Zealand.

[3]Nuffield Department Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom.

The contribution of vascular endothelial growth factor (VEGF) family members to neovascularisation and metastasis in human colorectal carcinoma (CRC) is unclear. We therefore measured the mRNA levels of VEGF-A isoforms (VEGF-A_{189}, VEGF-A_{165}, VEGF-A_{121}), VEGF-B, VEGF-C, and their tyrosine kinase receptors VEGFR1 (Flt-1), VEGFR2 (KDR), and VEGFR3 (Flt-4) in normal colon (n=20), adenoma (n=10), and invasive colorectal carcinoma (n=71) samples by RNase protection assay (RPA), and correlated expression levels with standard clinicopathological variables. VEGF-A and VEGF-C mRNA levels were positively correlated with tumour grade and tumour size ($p<0.05$), but not patient age, sex, presence of infiltrative margin, lymphocytic response, vascular invasion, Duke's stage, or node involvement ($p>0.05$). VEGF-B mRNA abundance showed a positive association with the presence of an infiltrative margin ($p=0.044$). Gene expression studies demonstrated differential regulation of VEGF ligands during adenoma-carcinoma progression. VEGF-A mRNA abundance was greater in adenoma and carcinoma samples compared with normal tissue ($p<0.05$), and VEGF-B mRNA levels were highest in adenoma samples ($p=0.012$). In contrast, VEGF-C mRNA abundance was higher in carcinoma than normal or adenoma tissue ($p=0.003$), with expression levels highest in invasive adenocarcinomas with lymph node metastases (Duke's Stage C). Flt-1 and KDR receptor mRNA levels were higher in adenoma and carcinoma compared to normal tissue ($p\leq0.001$), whereas Flt-4 mRNA levels appeared lower in carcinoma samples. These data support the hypothesis that VEGF ligands undergo an angiogenic switch at different stages during progression from normal colon to invasive CRC: VEGF-A and VEGF-B initiate and sustain angiogenic responses, whereas VEGF-C promotes invasive tumour growth and lymph node metastasis.

Patterns of alcohol use and misuse among elderly rest home residents in Christchurch, New Zealand

Nadim Khan[1], Tim J Wilkinson[1], Douglas J Sellman[2], Patrick Graham[3]

[1]Health Care of the Elderly, Department of Medicine, [2]Department of Psychological Medicine and [3]Department of Public Health and General Practice, Christchurch School of Medicine and Health Sciences, University of Otago, New Zealand

To determine the prevalence of alcohol use and misuse among elderly rest home residents in Christchurch, a cross-sectional prevalence survey was conducted among 175 residents aged 65 and over, randomly selected from 30 rest homes in Christchurch, New Zealand, in 1998. Hazardous patterns of alcohol consumption in the past 12 months were determined by the Alcohol Use Disorders Identification Test (AUDIT) questionnaire and alcohol dependence in the past 12-months and lifetime was determined by a structured clinical interview using DSM-IV criteria.

Of 246 eligible participants, 175 (71.1%) residents were interviewed, 115 women and 60 men. The mean age of participating residents was 82.6 years (SD=7.8) compared with 83.2 years (SD=6.3) for non-participants in the study.

The prevalence of hazardous patterns of alcohol consumption in the past 12 months by the AUDIT (cut-off score 8) was 5.1% (95% CI=1.8-8.4). According to DSM-IV criteria, the prevalence of lifetime alcohol dependence was 20.5% (95% CI = 13.5-27.6). The prevalence of alcohol dependence in the past 12 months was 0.5% (95% CI = 0-1.7). The prevalence of lifetime alcohol dependence was significantly higher in men 36.7% (95%CI = 23.2-50.1) than women 12.2% (95% CI = 5.6-18.8) (p=0.0001).

In spite of advanced age, a small proportion of elderly rest home residents consumed quantities of alcohol that puts them at risk of future damage to physical or mental health. Lifetime prevalence of alcohol dependence was comparable to the general population estimates and was higher in men than women.

Pressure controlled ventilation compared with volume controlled ventilation in anaesthetised adult patients

RA French

Department of Anaesthesia, Christchurch Hospital, Christchurch, New Zealand

Ventilators for use in the Intensive Care Unit have been able to offer a variety of ventilatory modes for some years. The introduction of anaesthesia "work-stations" now allows the anaesthetist a choice of ventilatory mode, principally volume vs. pressure control ventilation. No comparative study of these modes of ventilation appears to have been performed in the anaesthetic setting.

This study examined patients undergoing general anaesthesia with the use of intermittent positive pressure ventilation. Ethics committee approval was gained and twelve patients studied. Pressure control (PCV) and volume control (VCV) modes (Datex Anaesthesia Delivery Unit) were used within the same patients in situations of stable respiratory mechanics. VCV and PCV modes were used sequentially and pressure, flow and volume waveforms, as recorded by sidestream spirometry (Datex AS/3), were captured using an analogue to digital converter and a computer.

Pressure control ventilation and volume controlled ventilation delivered a clinically equivalent tidal volume and peak airway pressure in all patients studied. In the majority of patients (11 of 12), PCV delivered a slightly greater tidal volume per cmH_20 applied peak pressure. The mean difference was 2.2 ml per cm H_20 applied ($p<0.001$, 95% CI 1.1 to 3.3 ml/cm H_20).

Pressure control ventilation offers a satisfactory mode of providing intermittent positive pressure ventilation in anaesthetised adults. It possesses a small advantage over volume control ventilation when considering the tidal volume delivered per unit of peak pressure. This may be of benefit when attempting to limit peak airway pressures whilst maximising tidal volume.

Oxidation of β_2-agonists by peroxidases and its relevance to asthma control

Stephen J. Hoskin, Anthony J. Kettle.

Free Radical Research Group, Department of Pathology, Christchurch School of Medicine and Health Sciences, PO Box 4345, Christchurch, New Zealand

β_2-agonists are useful for dilating airways in acute episodes of asthma but regular use may worsen asthma and cause tolerance. The aim of this study was to investigate a biochemical mechanism that could explain such findings.

Using absorbance spectroscopy, we investigated whether β_2-agonists act as substrates for peroxidases – the enzymes released by eosinophils and neutrophils during inflammatory processes like asthma. Fenoterol, isoproterenol, terbutaline, salbutamol and formoterol, were all capable of reacting with myeloperoxidase (MPO) and eosinophil peroxidase (EPO.) Fenoterol and isoproterenol showed highest affinity whereas salbutamol was a very poor peroxidase substrate.

Oxidised terbutaline formed several new products which we detected using high performance liquid chromatography (HPLC.) Some products were fluorescent and showed an absorbance spectrum characteristic of phenol dimers. Oxidation of terbutaline also produced a terbutaline peroxide, measured using the ferrous oxidation of xylenol (FOX) assay. Isolated neutrophils and eosinophils oxidised terbutaline to form the same range of products as isolated enzyme.

In conclusion, β_2-agonists are oxidised by peroxidases in the presence of hydrogen peroxide. Drug affinity for peroxidase roughly correlates with adverse effects. Closer investigation of terbutaline revealed that oxidation occurs via a free radical pathway to produce multiple products including phenol dimers and peroxide. β_2-agonist administration into the inflammatory environment of an asthmatic airway could result in products capable of increasing inflammatory damage in the lung. The β_2-agonist could also be inactivated. Our investigations confirm that inflammatory cells are capable of oxidising terbutaline. Further studies are needed to identify whether β_2-agonist oxidation occurs *in vivo.*

Patient agitation and heart rate variability

ZH Lam, S. Hunt, J. Geoffrey Chase[1], Geoff Shaw[2]

[1] University of Canterbury, Department. of Mechanical Engineering, PO Box 4800, Christchurch, New Zealand

[2] Departmment of Intensive Care Medicine, Christchurch Hospital, Christchurch, New Zealand

The dynamics of agitated patients are not understood and the resulting over-sedation has a high social cost and risk to the patient. This research aims to develop sensor arrays and signal processing systems to quantify the agitation response of sedated and ambulatory patients.

Data from continuously monitored electrocardiographs ECG's were taken from a lightly sedated, mildly agitated critically ill patient, and from two normal ambulatory subjects. The normal subjects also underwent a cold compress test in order to simulate physiological effects of agitation. The signal 'noise' of the ECG was reduced with the use of a moving average filter before determining the RR-intervals. The RR-intervals were then analysed for heart rate variability (HRV).

Our results show that although the overall heart rate does not increase significantly with agitation, the RR-intervals have higher power content in the 0.4-0.5Hz high frequency range. During the cold pressor test spectral power around the low frequency (0.05Hz) band reduces approximately 5dB transferring to peaks over 10dB greater in the high frequency (0.4-0.5Hz) band. These results show the potential for determining the state of agitation employing Fourier analysis and Autoregressive modelling of the RR-interval.

It is hoped this research will enable better patient care, create commercial opportunity in medical devices and systems and provide a quantifiable technology platform for assessing the efficacy of a wide range of sedation therapeutics.

Sialic acid content of fibrinogen in pregnant women and in individuals on fibrate therapy

G. Maghzal, S. Brennan and P. George

Molecular Pathology, Canterbury Health Laboratory, Christchurch, New Zealand

Fibrinogen is a plasma glycoprotein, which plays a pivotal role in blood coagulation, and has been associated with a higher risk of cardiovascular disease and thrombosis. A distinct correlation has been made between the sialic acid content of fibrinogen and the thrombin clotting time (TCT) of plasma. For example, acquired dysfibrinogenemias caused by liver disease have an increased sialic acid content of fibrinogen and a significant delay in TCT.

We have studied the sialic acid content of fibrinogen in two populations: 1) pregnant women, who reportedly have increased fibrinogen and sialic acid levels and an increased risk of thrombosis; and 2) individuals on fibrates, which are used to control dyslipidaemia and have been shown to decrease the functional level of fibrinogen.

Using electrospray ionisation mass spectrometry, we found a 5.4% increase in the sialic acid content of fibrinogen ($p < 0.01$) in the fibrate population (n=12) compared to normal controls (n=6). While in the pregnant women who were in the third trimester (n=11), there was a 5.5% decrease in the sialic acid content of fibrinogen ($p < 0.05$). Pregnant women also had significantly higher fibrinogen levels and lower TCT. We also measured the thrombin-catalysed polymerisation rate of these fibrinogens and found that the fibrates group had a significantly lower rate (V_{max}) compared to the controls (5.6×10^{-4} s^{-1} vs 6.6×10^{-4} s^{-1}) while this rate was increased in the pregnant women. These findings strongly suggest that the sialic acid content of fibrinogen affects its clotting and may contribute to the increased risk of thrombosis in pregnant women and the decrease of functional fibrinogen during fibrate therapy.

C-type natriuretic peptide (CNP) and amino terminal pro C-type natriuretic peptide (NT-proCNP) in a sheep model of mild sepsis

Timothy C.R. Prickett[1], Timothy G. Yandle[1], Graham K. Barrell[2], Martin Wellby[2], M Gary Nicholls[1], Eric A. Espiner[1] and A. Mark Richards[1].

[1] Department of Medicine, Christchurch School of Medicine and Health Sciences, Christchurch, New Zealand.

[2] Animal & Food Sciences Division, Lincoln University, Canterbury, New Zealand.

CNP is a peptide hormone synthesised in the brain, vascular endothelium and bone, where it functions as a neurotransmitter, vasorelaxant and stimulator of long bone growth respectively. We have identified a circulating peptide from the amino terminal end of the precursor proCNP in human and sheep plasma. This peptide has an apparent molecular weight of 5 kDa, similar to that expected for NT-proCNP(1-50) – a potential fragment released during processing of proCNP. However the relation between the two forms, and the source of the immunoreactive forms found in plasma are unknown. In health, plasma levels of CNP are close to detection limits and only in the setting of sepsis has elevation of CNP been observed *in vivo*. Since endothelial cells stimulated by lipopolysaccharide (LPS) release CNP *in vitro* we aimed to see if CNP levels were raised in an animal model of mild sepsis and whether plasma concentrations correlated with NT-proCNP.

Sixteen sheep received an i.v. bolus of LPS (800ng/kg live weight) or vehicle. Changes in rectal temperature at 4 h were –0.2 $\pm$ 0.1 ^{0}C (mean $\pm$ sem) for control sheep, and +1.1 $\pm$ 0.2 ^{0}C for LPS treated sheep. LPS induced a rise in plasma CNP at 2 h (P<0.005), and NT-proCNP at 3 h compared with control sheep. CNP and NT-proCNP plasma concentrations were significantly correlated (R=0.44, P<0.0001).

These results support the hypothesis that CNP and NT-proCNP are released from the same source during the processing of proCNP. Plasma measurements of the novel peptide NT-proCNP, which circulate at higher levels than CNP, opens the possibility of studying factors regulating CNP *in vivo.*