WAIKATO MEDICAL
RESEARCH FOUNDATION

Discovery, Innovation, Progress.

2015 ANNUAL REPORT

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CHAIRMAN'S REPORT

It is with great pleasure that I write this Chairman's Report. When I became Chairman twelve or so years ago, I knew that my main purpose would be to do something about fundraising. We had a number of aborted attempts at this because none of the trustees are fundraisers, or marketers, and so eventually we had the wisdom to engage a professional fundraiser, Russell Mayhew, who has been remarkably helpful to us in fundraising – and I highly recommend him. He was highly professional, and he has developed a Corporate Team of Influence, a Medical Team of Influence, and by doing this, we have been very successful in raising firstly, our profile in the community, and secondly, our funds.

'WE HAVE BEEN VERY SUCCESSFUL IN RAISING FIRSTLY, OUR PROFILE IN THE COMMUNITY, AND SECONDLY, OUR FUNDS'

A summary of our fundraising @ 15 August 2015

TOTAL :	$1,638,590
Public Donations:	$514,863
Foundation Trustees:	$148,340
Medical Field/Payroll:	$559,290
Launch/Panmedia Golf Day/Gallagher Dinners:	$121,000
Regional Committee:	$16,000
Corporation Committee:	$55,500
Other Trusts:	$223,597

Fundraising is ongoing. Early in the fundraising, there was some cynicism expressed regarding whether the doctors would come to the party, and we see that he donations from doctors, either as donations or through the payroll, have been very well attended to.

Of course, we are also very grateful to Trust Waikato who continues to be our main supporter, and has been for at least twenty years.

During the fundraising, we have managed to find other sources of funding, and this includes the Cancer Society (which has been a regular supporter for a number of years), Braemar Charitable Trust, Rodmor Charitable Trust, and Grassroots Trust.

I would like to express my great gratitude to the helpful advice and encouragement given to us by our Patron, Dr Peter Rothwell, who has unfortunately had to resign due to ill health, and also to the many people who have helped in our fundraising, who are very numerous – but one or two need mentioning, and I think particularly of Brian King (Harcourts), Peter De Luca (Tompkins Wake) and Murray Day, who have been tireless workers raising our profile, and encouraging donations.

Once again, our Grants Committee has been very active at screening applications for funding and this year was another year where we found some of the grants weren't up to scientific standard, so they have been declined money – which has allowed us together with our fundraising appeal over the last three years to have a second grants round, hoping to flush out some young investigators.

We are also very happy to welcome onto the Grants Subcommittee Dr Deborah Harris, New Zealand's first nurse practitioner, who has now completed a PhD through the Sugar Babies Study which we are very proud that we have supported.

Finally, the reason I am overjoyed to do this report this year is that it will be my last report as Chairman, and I am pleased to say that I think I have left the Foundation in a very good position to go forward, as I know we have some very committed trustees, and that one of the strengths of our Foundation is the very little amount of money we spend on expenses, and that all the trustees are volunteers. I wish the Foundation all the very best in future years, and will be maintaining contact initially as Patron.

Dr Noel Karalus

GRANTS CHAIRMAN'S REPORT

This year, the WMRF received 11 applications requesting a total of $203,000. Due to the efforts of the fundraising committee and welcome financial assistance via sponsorship grants from Trust Waikato, Grassroots Trust, Waikato BOP Division of the Cancer Society, and the Noel Karalus Respiratory Research Scholarship, the committee recommended supporting 9 applications, listed in no particular order in the table below, naming just the lead investigators.

This year, not all available funds were allocated so it was decided, for the first time, to hold a second small grant round toward the end of this year. This gives an opportunity for unsuccessful applications to be revised and resubmitted. Applications from young investigators are also encouraged.

WMRF FUNDED PROJECTS GRANT ROUND 2015	
• *Glucose in Well Babies (GLOW)* Dr Deborah Harris, Neonatal Nurse Practitioner, Waikato Hospital, Hamilton	$30,060
• *Effect of general anaesthetics on spatial patterns of field potential activity recorded from mouse neocortical brain slices* Dr Logan Voss, Department of Anaesthesia, Waikato Hospital, Hamilton	$17,270
• *Circulating MicroRNAs as a biomarker of thyroid cancer* Dr Goswin (Win) Meyer-Rochow, Department of Surgery, Waikato Hospital, Hamilton	$20,453
• *Title Phase 1b pharmacokinetic (PK) and pharmacodynamic (PD) trial to identify the optimal selenium (Se) compound for use with cancer therapies* Dr Stephen (Steve) Evans, Pharmacy Department, Waikato Hospital, Hamilton	$30,000
• *Automatic Oxygen Delivery for Regulation of Patient Blood Oxygen Saturation Level* Dr Jonathan Scott, University of Waikato, Hamilton	$30,000
• *The Role of PhoH2 in the pathogenesis of Mycobacterium tuberculosis* Dr Emma Andrews, University of Waikato, Hamilton	$19,720
• *Painbuster rectus sheath infusion device for analgesia following laparotomy* Dr Kelly Byrne, Anaesthetic Department, Waikato Hospital, Hamilton	$10,400
• *Determining the molecular pathways responsible for the anti-mycobacterial effects of NSAIDS (non-steroidal anti-inflammatory drugs)* *Dr Ray Cursons, University of Waikato, Hamilton*	$19,500

I thank the Grants Committee (Maggie Fisher, Amanda Oakley, two new members : Vic Arcus and Deborah Harris) the WMRF Chairman, Noel Karalus and Ian Jennings, Finance Committee Chairman for reviewing, scoring and ranking the applications. Michael Jameson was on overseas sabbatical leave during the review process; hence unavailable as a reviewer in this round but whose valuable input is appreciated.

A special thank you goes to the WMRF administrator Robyn Fenneman for gathering, organising and meticulously presenting the applications to the committee and responding to applicants.

Dr Adrian Molenaar
Chair, Grants Committee

MEET TWO NEW MEMBERS TO OUR TEAM AT THE FOUNDATION

VIC ARCUS, PROFESSOR MOLECULAR BIOLOGY, UNIVERSITY OF WAIKATO

Vic is a professor in biology at the University of Waikato. He leads a group of researchers looking at the molecular biology of tuberculosis. Tuberculosis has been described by the World Health Organisation as a global epidemic. It kills more people every year (~3 million) than any other single infectious disease. New Zealand has relatively low incidence rates of tuberculosis but vigilance is still required even here.

Vic did his undergraduate science degree at the University of Waikato and then flew off to Cambridge UK to do a PhD in molecular biology. He then spent eight years at Auckland University before returning "home" to Hamilton.

Vic likes music and football (soccer). He likes to swim, bike and run. Up until recently, he and his wife Fiona, undertook these activities with their two kids, Leon and Grace. Unfortunately for Fiona and Vic, the kids have got faster and they have got slower.

'tuberculosis kills more people every year (~3 million) than any other single infectious disease'

DEBORAH HARRIS, NEONATAL NURSE PRACTITIONER

Following her nurse registration in Wellington in 1985, Deborah Harris initially worked in oncology and haematology.

"I then headed over to England, where I worked at Harefield Hospital in Middlesex, which specialised in heart and lung transplants in adults," she says.

However, her work focus – and her life – changed forever when the hospital became desperately short-staffed in the Paediatric Intensive Care Unit. "I had been working in the unit for less than a day when I knew that I would never go back to working with adults. It was so incredibly special to work with people that are becoming families, and it still is."

In 2002 Deborah Harris became New Zealand's first nurse practitioner, and has practiced as such in Waikato Hospital's Neonatal Intensive Care Unit since then. She says the role of a nurse practitioner brings a blend of nursing, science and medicine to the bedside. "I think that blend can make a real difference to a patient's experience."

Despite a national-first, a Master's degree and being a highly skilled health professional, she was soon ready for another challenge. In 2013 she completed a PhD with her thesis on neonatal hypoglycaemia as part of the Sugar Babies study which looked into the common condition of neonatal hypoglycaemia (low blood pressure in babies).

Accolades for the Waikato nurse practitioner behind the life changing Sugar Babies study just keep coming. Dr Deborah Harris' thesis on neonatal hypoglycaemia has received a University of Auckland's Vice Chancellor's Prize for Best Doctoral Thesis in 2013. Dr Harris' thesis was one of just five awarded the prize out of 321 doctoral theses completed in 2013 and one of 18 nominated for the top prize. Her thesis was nominated by the Faculty of Medical and Health Sciences, on behalf of Auckland University's Liggins Institute where the research was carried out. The study took place in Waikato Hospital's Neonatal Intensive Care Unit where Dr Harris works as a neonatal nurse practitioner.

"I wanted the focus of my study to be something that would benefit babies and their mums," she says.

'a nurse practitioner brings a blend of nursing, science and medicine to the bedside'

SUMMARY OF FUNDED PROJECTS FROM GRANT RECIPIENTS 2015

GRANT #243 // DR DEBORAH HARRIS, NEONATAL NURSE PRACTITIONER, WAIKATO HOSPITAL

Glucose in Well Babies (GLOW)

Neonatal hypoglycaemia is important because it is common and linked with brain injury and poor neurological outcome. In recent years, there has been considerable interest in the detection and management of neonatal hypoglycaemia. Newborns who are identified as being at risk are screened using heel-prick blood tests for the first days after birth. If hypoglycaemia is diagnosed, then treatment is usually provided. Glucose is the primary cerebral fuel and the aim of treatment is to increase the blood glucose concentration to ensure adequate cerebral energy supply.

The definition of neonatal hypoglycaemia has caused considerable controversy. The current accepted definition < 2.6mM has been determined using limited, but the only available data. However, the normal glucose profile of healthy appropriately grown term newborns has never been reliably described, and it is possible that many babies are being unnecessarily treated. Babies have been shown to use alternative cerebral fuels, primarily lactate and ketones. However, the normal concentrations of both blood lactate and ketone in healthy newborns within the first week are also unclear.

Aims

To describe the normal glucose, lactate and ketone profile in healthy newborns within the first five postnatal days.

Trial Design and Methods

We propose a prospective observational cohort study. We will meet families and seek informed consent prior to the birth. Eligible babies will be healthy, term and appropriately grown. Babies will be excluded if they are unwell for any reason or born to mothers who suffer from diabetes, obesity, or using medications that may influence blood glucose concentrations. Soon after birth, a blood test will be taken from the umbilical cord for the measurement of glucose, lactate and ketone concentrations. All subsequent blood tests will be by heel-prick lances. On the first day, these will match the frequency of current screening protocol for babies born at risk of neonatal hypoglycaemia and then continue for the next four days (maximum of 16 heel-prick blood tests). In addition, soon after birth a continuous interstitial glucose monitor will be placed subcutaneously in the baby's thigh. Parents will collect information about every feed on a small hand-held computer tablet. Babies will be cared for in their own homes.

Expected Benefits and Significance

Presently, there is no reliable description of the normal glucose, lactate and ketone profiles in the first week after birth. However, babies identified as being at risk for hypoglycaemia soon after birth, most of whom are otherwise healthy, are usually treated for hypoglycaemia if the blood glucose concentration is < 2.6mM. Furthermore, there is no evidence that treating transitional neonatal hypoglycaemia improves the neurological outcome for children, or adolescents.

Importantly, this study will describe the blood concentrations of glucose, lactate and ketone in healthy newborns, for the first time. If this study shows that "hypoglycaemia" as it is currently described is common in healthy babies, it is possible that the current management of neonatal hypoglycaemia will alter considerably, as fewer babies may be screened and treated for hypoglycaemia. This will have significant implications for babies, their families and health care resources.

GRANT #247 // PROFESSOR JONATHAN SCOTT, FACULTY OF SCIENCE AND ENGINEERING, THE UNIVERSITY OF WAIKATO

'a prototype will convince a local manufacturer to apply for MBIE funding to bring the device to market'

Automatic Oxygen Delivery for Regulation of Patient Blood Oxygen Saturation Level

This project aims to automate oxygen flow regulation economically in the regular hospital environment to improve patient outcomes and save operating costs.

British Thoracic Society oxygen guidelines (O'Driscoll et al, 2008) recommend that oxygen only be administered in response to measured low blood-oxygen saturation. In other words, oxygen should be administered only when it is needed. Currently, oxygen dosage is titrated manually by nursing staff, repeatedly adjusting flow rates and delivery devices. Audits have consistently shown that as a result of this, titration often does not happen appropriately, leaving the patient exposed to incorrect concentrations of oxygen, and wasting tens of thousands of dollars per hospital per annum. Feedback-controlled oxygen titration has been previously demonstrated with deep sea divers (Taplin, 1973) and neonates (Morozoff, 1993, Sun et al., 1997) or as a general system (Raemer 1994, Azhar and Karim, 1991) including recent work for highly-specific implementations (Verghese et al., 2013). Nevertheless, none of these capabilities ever migrated to a cost-effective, daily-use device for purchase and routine use in hospitals.

This project will build an implementation around existing New Zealand hospital equipment and infrastructure, producing prototype units that can be used to verify the effectiveness, and embodying a design that can be put into production by a suitable, hopefully-local, manufacturer. The expectation is that a prototype will convince a local manufacturer to apply for MBIE funding to bring the device to market.

GRANT #239 // DR EMMA ANDREWS, FACULTY OF SCIENCE AND ENGINEERING, THE UNIVERSITY OF WAIKATO

'… determining whether these proteins may pose as a suitable candidates for drug design'

The role of PhoH2 in the pathogenesis of *Mycobacterium tuberculosis*

The causative agent of tuberculosis in humans is *Mycobacterium tuberculosis* and in order to persist in its host, this organism must endure a number of stressors such as nutrient stress and hypoxia. The genome of *M. tuberculosis* is unique amongst pathogens for the number of toxin-antitoxin genes it encodes – a total of 85 at last count. A large proportion of these belong to the VapBC type II toxinantitoxin array in which VapC acts as a toxin and VapB its cognate antitoxin. These proteins, based on their biochemical activity, are hypothesised to be associated with the stress response and persistence of this organism. The one remaining VapC in M. tuberculosis belongs to the PhoH2 superfamily. PhoH2 proteins are prevalent amongst bacteria, and we have recently carried out the first investigation on these proteins by biochemically characterising this protein from *M. tuberculosis* and *Mycobacterium smegmatis*. PhoH2 differs from VapC toxins in that it encodes a toxin fused with what we identified as an RNA unwinding protein. Our study also established that PhoH2 in mycobacteria encompasses a cognate antitoxin (PhoAT).

This result leads to the intriguing possibility that PhoAT-PhoH2 may play a similar role to VapBC genes, likely contributing to the tight-knit metabolic control of this organism. Part of our study involved the making of a phoH2 deletion strain in *M. smegmatis* and we propose to extend our preliminary phenotypic research using this strain to determine the biological role of this intriguing group of proteins in mycobacteria. Deducing the biological role of this protein within pathogenically relevant organisms will provide understanding not only of what these proteins do in the organisms they reside but contribute to the broader understanding of *M. tuberculosis* biology and pathogenesis, determining whether these proteins may pose as a suitable candidates for drug design.

GRANT #241 // DR RAY CURSONS, FACULTY OF SCIENCE AND ENGINEERING, THE UNIVERSITY OF WAIKATO

Determining the molecular pathways responsible for the anti-mycobacterial effects of NSAIDS (Non-steroidal anti-inflammatory drugs)

Mycobacterium avium (M. avium) is becoming the most prevalent *Mycobacterium* infection in western countries. *M. avium* is an opportunistic pulmonary pathogen, infecting patients who often are vulnerable due to pre-existing medical conditions. Antibiotic therapy of *M. avium* is long (generally 18 months) and often unsuccessful at eliminating the infection, promoting resistance to commonly used antibiotics, such as clarithromycin.

M. avium exists as a biofilm in lung infections, adding to the difficulty in eradicating the infection. Our research investigated potential *M. avium* biofilm dispersing agents, as well as antibiotic synergy for improved *M. avium* treatment options.

Our findings show that ten clinically isolated *M. avium* samples were all highly resistant (>16µg.mL) to antibiotics commonly used in the treatment of *M. avium* infections, both in planktonic and biofilm phenotypes. *M. avium* isolates differed in biofilm phenotype, and correspondingly, their susceptibility to antibiotics and potential biofilm dispersing agents.

The addition of 3.6mg/mL (acetylsalicylic acid) or 3.6mg/mL Ibuprofen to planktonic and biofilm cells showed significant increase in *M. avium* cell death. Previously identified biofilm dispersing agents, such as mannitol, N-acetyl-L-cysteine, active Manuka honey, methylglyoxal, Lipitor®, 2,4-dinitrophenol and EDTA, had no significant effect on the MICs of the tested antibiotics against *M. avium*.

A concentration of 4mg/mL aspirin combined with 8mg/mL of EDTA sterilized and completed eradiated *in vitro* three-week old mature *M. avium* biofilms. In contrast these biofilms were completely resistant to all 8 antibiotics at concentrations of > 16µg.mL.

We propose to investigate the molecular pathways responsible for the anti-mycobacterial activity of NSAIDS in order to understand their activity. Furthermore we propose to investigate alternative biofilm dispersing agents, such as DNAse I (Pulmozyme) that have the potential to disrupt biofilms and thus reduce the MICs of currently used antibiotics.

'ten clinically isolated *M. avium* samples were all highly resistant to antibiotics'


SCIENTIFIC RESEARCH CONSISTS
IN SEEING WHAT EVERYONE ELSE
HAS SEEN, BUT THINKING WHAT
NO ONE ELSE HAS THOUGHT.

PAST GRANT RECIPIENTS FINAL REPORTS AND FINDINGS 2011 – 2014

FINAL REPORT ON GRANT #225 // MICHÈLE R PRINSEP DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WAIKATO

'to identify target or lead compounds for pharmaceutical/medical use or as research tools'

Specific Aims of the Project

At the University of Waikato, Drs Michèle Prinsep and Ryan Martinus and their students undertook a project with the following specific aims:

i) To collect a wide diversity of New Zealand bryozoan species from different geographical locations, with recollection of bioactive species where necessary.
ii) To collect New Zealand species of cyanobacteria, including those which cause "algal blooms", and to culture these if required.
iii) To screen extracts of collected species for a range of biological activities and to use these data in conjunction with LC-MS information, to identify samples of further interest.
iv) To utilise biological assays to assist in the isolation of bioactive compounds from bryozoans and cyanobacteria.
v) To characterise new biologically active compounds from bryozoans and cyanobacteria, especially those which represent new structural types.
vi) To explore the mode of action of bioactive metabolites through a secondary screening process and to carry out further studies as appropriate.
vii) If possible, to prepare analogues of bioactive natural products and test these for biological activity, thus finding which sub-structures of the original compound are responsible for the observed activity.
viii) Based on these findings, to identify target or lead compounds for pharmaceutical/medical use or as research tools.

Method

Marine organisms, especially invertebrates such as bryozoans were collected by SCUBA diving at various geographical locations around New Zealand, by our team of local divers. The Bay of Plenty was an area of particular focus, given its geographical proximity to Waikato and the fact that organisms from this region have not been widely studied for their secondary metabolites. Cyanobacterial samples, predominantly from "algal blooms" were collected and either cultured at the University of Waikato or at the Cawthron Institute, Nelson.

Crude extracts of marine samples were prepared and these extracts were then screened for biological activity against HeLa cells, two bacteria and one fungus using well-established methods. The samples were also screened using liquid chromatography-mass spectrometry (LC-MS) to obtain a chemical profile of all extracts under standard conditions and to assist in rapid identification of known metabolites (dereplication). LC-MS and Matrix Assisted Laser Desorption Ionisation-Time Of Flight (MALDI-TOF) mass spectrometry were used to screen cyanobacteria both for dereplication and to identify samples which potentially contained new compounds.

Extracts were then prioritised for further study based on their chemical and biological profiles and those deemed worthy of further attention by these means were investigated to isolate the source of the observed biological activity.

Pterocellin A, a potent cytotoxic metabolite of the marine bryozoan Pterocella vesiculosa was isolated previously by our group. The cytotoxic mode of action of this compound was examined in depth using the human cervical cancer cell line HeLa. Characteristic markers of apoptotic and necrotic cell death were investigated using several in vitro biochemistry techniques, including (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) bioassays. Cell viability was assessed using a trypan blue exclusion experiment and cells were examined under the microscope for any distinct morphological changes characteristic of apoptosis. HeLa cells treated with pterocellin A were stained with a mitochondrial specific fluorescent probe and visualised by a laser confocal fluorescent microscope. Other markers such as caspase-3 activation and DNA fragmentation were also investigated. The activity of caspase-3 was measured by FITC fluorescent staining and DNA fragmentation by extraction of DNA followed by 2% agarose gel-electrophoresis.

Results

Biological and chemical screening of marine samples identified a number of extracts of interest. In some cases, an extract which yielded promising bioassay results was discounted from further study as its taxonomic identification and chemical profile indicated that the compound/s responsible for the bioactivity were already known. Investigation of the remaining extracts of interest is ongoing (and will continue to be so as samples are collected and screened). Screening of cyanobacterial extracts similarly resulted in identification of some know bioactive metabolites but during the course of the time covered by the research grant, a number of new, bioactive metabolites were isolated and characterised from marine organisms and from

cyanobacteria. These compounds included the antimicrobial alkaloid 7-bromo-1-ethyl- -carboline from the marine bryozoan *Pterocella vesiculosa* and a number of new microcystins from various species of cyanobacteria, including some glycine-containing microcystins from the McMurdo Dry Valleys of Antarctica (see list of publications for relevant papers). A number of very promising leads from the marine samples are also currently being pursued.

The MTT bioassay indicated that pterocellin A is very effective at killing HeLa cells. Morphological changes in the HeLa cells were observed under the microscope after six hours of treatment with pterocellin A. Cell shrinkage and nucleus condensation were noted, as well as apparent membrane blebbing, a key feature of apoptosis.

A low level of cytosolic LDH was detected in the supernatant after all the cells had died from pterocellin A treatment at high concentration. A trypan blue exclusion cell count was carried out after treating cells with different concentrations of pterocellin A for 24 hours. It was found that no stained cells were observed after treatment and the cell number steadily decreased as the concentration of pterocellin A increased.

When HeLa cells were exposed to pterocellin A (at 1000 ng/ml) there was a re-distribution of mitochondria from the cell cytoplasm to the peri-nuclear region after 6 hours. Compared to healthy mitochondria that have an elongated tubular formation, the mitochondria of treated cells appeared to be fragmented and globular. The redistribution of and morphological changes in the mitochondria took place before any notable changes in cell morphology overall and cell death (as indicated by the formation of membrane blebbing/apoptotic body formation).

Upon treatment with pterocellin A, the level of activated caspase-3 increased by approximately two-fold compared to the control. Faint laddering (indicative of fragmentation) was detected by agarose gel-electrophoresis for DNA extracted from pterocellin A-treated cells and from cells treated with the positive control rotenone.

This project also utilised the biochemical assays to develop a systematic bioassay screening system at the University of Waikato, and further investigated the bioactive metabolites of *P. vesiculosa*. The MTT and LDH bioassays were applied to bioactive fractions separated from the crude extract. These fractions were analysed by LC-MS and several unknown metabolites were observed, however due to time constraints associated with the project, no further investigation has been carried out to date.

Discussion

Cellular toxicity can be manifested as either necrotic or apoptotic cell death. Cancer cells are prone to resisting apoptosis, and one of the key mechanisms recognised in anticancer agents is the induction of apoptosis.

Most of the above results indicate that the bryozoan metabolite pterocellin A induces apoptosis in HeLa cells. Morphological changes in the cells after treatment with pterocellin A such as shrinkage, nuclear condensation and membrane blebbing are consistent and characteristic features of apoptosis. Both the LDH bioassay and the trypan blue exclusion cell count experiments indicated that the cells maintained membrane integrity. In necrotic cell death, the cell membrane ruptures leaking cellular contents into the surroundings. Only a low level of cytosolic LDH was detected in the supernatant after all the cells had died from pterocellin A treatment at high concentration, indicating that the cells maintained membrane integrity upon death. A basic trypan blue exclusion cell count can distinguish between dead and live cells. The lack of stained cells observed after pterocellin A treatment indicate that the plasma membranes were intact, again indicative of apoptosis. Yet another piece of evidence supporting the apoptotic mechanism of cell death was the activation of caspase-3 in HeLa cells after treatment by pterocellin A. Activated caspase-3 can only be detected after a series of signalling events following the induction of apoptosis.

The MTT data were indicative of mitochondria impairment which could suggest that these are a target of the metabolite. This idea was supported by the fact that changes in morphology and location of the mitochondria after exposure to pterocellin A occurred before any cell morphological changes indicative of apoptosis. DNA fragmentation is one of the most well-known characteristics of apoptosis and while it was observed for pterocellin A-treated cells and for the positive control rotenone, the method requires some further refinement for optimisation of results.

All of the above data strongly indicate that pterocellin A exerts its cytotoxicity in HeLa cells via apoptosis involving mitochondrial processes. The mitochondrial signalling pathways responsible have not yet been investigated, and will be the subject of a future grant application to the WMRF.

To date, results of all of the above work have been disseminated in the following ways:

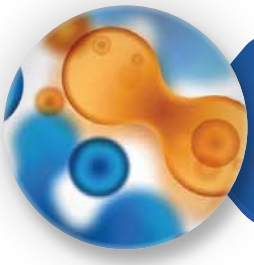
Publications:

• Article in Refereed Journal

J. Puddick, M. R. Prinsep, S. A. Wood, S. C. Cary, D. P. Hamilton and P. T. Holland, Further characterization of glycine-containing microcystins from the McMurdo Dry Valleys of Antarctica.
Toxins, 2015, 7, 493-515.

J. Wang, M. R. Prinsep, D. P. Gordon, M. Page and B. R. Copp, Isolation and Stereospecific Synthesis of Janolusimide B from a New Zealand Collection of the Bryozoan *Bugula flabellata. J. Nat. Prod.*, 2015, 78, 530-533.

FINAL REPORT ON GRANT #185 // DR RODRIGO BIBILONI AGRESEARCH, RUAKURA RESEARCH CENTRE



'Our results contribute towards the understanding of how bacteria can influence our health'

Introduction

Epidemiological studies have shown that growing up on a farm is associated with a lower risk of allergy and the consumption of raw milk is strongly correlated with this effect. The interaction of bacteria with our immune system is a crucial element in determining the balance between allergy, inflammation, and tolerance. The hypothesis of our study was that a mechanism of bacterial trafficking from the gut to the mammary previously reported in lactating mothers may also occur in the cow, possibly contributing to the bacterial components found in raw milk. In addition, the identification of bacteria or bacterial components in unprocessed cow's milk and comparison to those in breast milk may provide the foundation for new strategies on how to shape the intestinal microbiota of the infant to aid in the prevention of allergic disorders.

Consequently, the study involved sampling faeces, blood and milk from animals, as well as recruiting human volunteers to collect the same type of samples. Bacteria within these samples were identified using next generation sequencing. Both parts of the study received ethics approval.

Development of experiments – collaborations

During the course of the experiments, we recruited a summer student from Victoria University under the Summer Scholarship Scheme (50% funds for stipend contribution from Victoria University, 50% contribution from AgResearch). The project plan attracted high interest from the Director of the Malaghan Institute, Dr Graham Le Gros, who supported the application for funds to complement those from the WMRF. A report from the student, Mr Richard Portch, is available to you if required.

Part 1 – Animal study

As indicated in the interim report (May 2012) we faced difficulties in obtaining bacterial DNA of sufficient amount and quality for sequencing. Although samples from animals were successfully collected and processed to separate the target cells (white blood cells from blood and milk), we were unable to detect sufficient bacterial signals using polymerase chain reaction (PCR), despite the use of several methods for extracting bacterial nucleic acids. This suggested that the bacterial load in the samples were much lower than expected from studies performed in humans (Perez et al, 2007), or that inhibitors were concomitantly extracted with the nucleic acids.

We therefore performed a second round of sample collection involving larger volumes of samples : 200 ml of blood and 800 ml of milk from 12 cows. Samples were collected in April 2013 after ethics approval was granted. Considering the challenge of collecting large volumes of samples in an aseptic fashion, we managed to confirm by means of microbiology controls that the conditions for collection were satisfied, and DNA of sufficient quality was able to be extracted and sequenced.

Our results showed that :

1. Bacterial DNA from milk and white blood cells was successfully extracted, amplified, sequenced and taxonomically identified.
2. A small proportion of the bacteria identified in faeces, milk, and white blood cells could be

found in all three sample types in some animals. This result supports the proposition that at least some of the bacteria present in milk may be the result of trafficking from the gut.

3. Bacterial profiles in the faeces, milk, and white blood cells were distinctive from one another. The faecal communities were more similar from cow to cow, compared to the milk and white blood cell communities.

Our results support the hypothesis that bacteria can be transported to the mammary gland from the gut through association with white blood cells. In particular, the high prevalence of bifidobacteria among milk cell bacteria is of relevance as numerous studies have shown that bifidobacteria can reduce symptoms of allergy and inflammation. In addition, the results show that next generation sequencing technologies can be successfully applied to detect the presence of bacteria in different sample types.

Part 2 – Human study

Human recruitment was conducted at the Waikato Hospital under the supervision of Dr Arun Nair. Unfortunately the recruitment of eligible volunteers was unsuccessful because mothers that were willing to donate their samples were unable to participate in the study due to antibiotic use. One of the selection criteria was that mothers had not been treated with antibiotics in the preceding weeks. Although we extended the recruiting to screen for participants from other Hospital Units we were unable to obtain any eligible volunteers, and we therefore decided not to progress this part of the study.

Conclusion and next steps

In this study, we have successfully identified bacterial groups that can be found in the faeces, white blood cells, and milk cells from the same lactating cow. Our results contribute towards the understanding of how bacteria can influence our health, and the mechanisms by which they do so. We believe that our results merit publication and we are in the process of preparing a manuscript. We will acknowledge WMRF as source of funds and we will send you a copy of such manuscript before submission. We thank the WMRF for providing the opportunity to perform this research.

FINAL REPORT ON GRANT #214 // DR KELLY BYRNE ANAESTHETIST, WAIKATO HOSPITAL

'Morphine or tramadol are equally efficacious in treating refractory pain in PACU'

Summary

This study aimed to discover whether tramadol or morphine was more effective at reducing pain scores for patients with ongoing pain in the post-anaesthesia recovery unit (PACU).

All eligible patients were recruited pre-operatively, and those with a pain score of six or more following 10mg of morphine in the recovery unit, were eligible to enter the study. They were then randomized to receive up to a further 10mg of morphine or 100mg of tramadol to control their pain.

There was no statistically significant difference in any of the outcomes measured. Mean length of recovery unit stay was 119 minutes in the morphine group and 120.4 in the tramadol group. 51% of patients were ready for discharge at the end of the study drug titration in the morphine group, and 53% of the patients in the tramadol group.

Morphine or tramadol are equally efficacious in treating refractory pain in PACU.

FINAL REPORT ON GRANT #191 // DR GOSWIN (WIN) MEYER-ROCHOW CONSULTANT GENERAL AND ENDOCRINE SURGEON, WAIKATO HOSPITAL

Background

'no previous studies determining whether SDH mutation-associated phaeochromocytomas demonstrate abnormal mitochondrial morphology'

Phaeochromocytomas are neuroendocrine tumours arising from chromaffin tissue. Although the majority are benign they are all potentially lethal due to the episodic secretion of large quantities of catecholamines (adrenaline and noradrenaline). These tumours are recognised to be highly vascular and metabolically active and contain cells densely packed with mitochondria. Succinate dehydrogenase (SDH) is an enzyme bound to the inner membrane of mitochondria. Succinate dehydrogenase subunit B (SDHB) germline mutations are associated with the development of phaeochromocytomas and paragangliomas and have a higher incidence of malignant disease than sporadic or the other familial phaeochromocytomas/paragangliomas.

Mitochondria have been implicated in the development of other tumour types however there have been few reports of regarding the ultrastructure of mitochondria in phaeochromocytomas and no previous studies determining whether SDH mutation-associated phaeochromocytomas demonstrate abnormal mitochondrial morphology. Given the close relationship of the SDH enzyme with mitochondria, we hypothesise altered mitochondrial morphology occurs in tumours from patients with SDHB germ line mutations.

Research Design

Electron microscopy (EM) was used to evaluate mitochondrial morphology on phaeochromocytoma tissue samples collected prospectively and preserved in Glutaraldehyde from 5 patients with SDHB germline mutations and from 5 patients with phaeochromocytomas without an SDHB germline mutation against the mitochondria from normal tissue (adrenal medulla).

Results

At the conclusion of the study 2 samples of normal adrenal medulla were collected (one from a patient with Conn's syndrome the other from a non-functioning adenoma), 5 SDHB associated tumours (2 from the same patient) and 4 other phaeochromocytoma (2 sporadic, 1 neurofibromatosis, 1 MEN2B). Electron microscopy revealed features consistent with active neurosecretory cells but no specific features that differentiated the mitochondria of SDHB associated phaeochromocytomas with either the normal adrenal medulla or other phaeochromocytoma tissue samples.

Conclusion

This study did not demonstrate any Electron Microscopic mitochondrial features in SDHB associated phaeochromocytomas that could differentiate from normal adrenal medulla or other phaeochromocytoma types. Thus EM is not a useful screening tool for the presence of an underlying SDH germline mutation.

FINAL REPORT ON GRANT #188 // DR MARIANNE ELSTON ENDOCRINOLOGIST, WAIKATO HOSPITAL

Somatostatin receptor subtypes in SDHB germline mutation positive and sporadic phaeochromocytomas and paragangliomas

Marianne S Elston[1,2], Goswin Y Meyer-Rochow[2,3], John V Conaglen[1,2], Anthony J Gill [4]Department of Endocrinology, Waikato Hospital, Hamilton 3240; [2]Waikato Clinical School, University of Auckland; [4]Department of Surgery, Waikato Hospital, Hamilton; [4]Department of Anatomical Pathology, Royal North Shore Hospital, Sydney 2065, Australia

'…it would suggest that octreotide therapy (unlabeled or radiolabeled) might have a therapeutic role in these patients'

Introduction

Many neuroendocrine tumours, including phaeochromocytomas and paragangliomas (PGLs), express one or more somatostatin receptors (SSTR) 1-5. A number of studies have reported SSTR expression in phaeochromocytomas and PGLs. However, receptor expression patterns have been conflicting and until recently specific monoclonal antibodies were not available against SSTR1-5. The aim of this study was to compare SSTR1-5 expression in *SDHB* germline mutation-associated phaeochromocytomas and PGLs with sporadic tumours.

Methods

Written informed consent was obtained from all patients. Immunohistochemistry for SSTRs 1-5 and *SDHA* and *SDHB* was performed using specific monoclonal antibodies on archived formalin-fixed paraffin-embedded tissue.

Results

Twenty-one tumours were included (13 phaeochromocytomas, 8 PGLs); 6 tumours were from patients known to have an underlying *SDHB* germline mutation (3 with malignant disease) and 15 were sporadic tumours. All *SDHB*-associated tumours had absent staining of *SDHB* on immunohistochemistry with preserved *SDHA* staining, consistent with the known *SDHB* germline mutation. All sporadic tumours demonstrated positive staining for both *SDHA* and *SDHB* consistent with the absence of an underlying *SDHA* or *SDHB* mutation. All *SDHB*-associated tumours demonstrated strong staining for SSTR2A. In contrast 9/16 sporadic tumour demonstrated weak or absent staining for SSTR2A. With the exception of one sporadic tumour, which demonstrated weak SSTR5 staining, all tumours had absent SSTR5 staining.

Conclusion

This study confirms the utility of using *SDHA* and *SDHB* immunohistochemistry to screen for an underlying *SDHx* germline mutation in patients with phaeochromocytoma/PGL. SSTR2A appears differentially expressed in *SDHB*-associated tumours as compared with sporadic tumours. If these findings are confirmed in a larger group of *SDHB*-associated tumours it would suggest that octreotide therapy (unlabeled or radiolabeled) might have a therapeutic role in these patients.

FINANCIAL STATEMENTS FOR THE YEAR ENDED 31 MAY 2015

THE FOLLOWING FINANCIAL STATEMENTS PROVIDE AN EXCERPT FROM THE AUDITED FINANCIAL STATEMENTS. THE UNQUALIFIED AUDIT REPORT WAS COMPLETED BY MARK CAMPBELL OF CAMPBELL AND CAMPBELL ACCOUNTING CONSULTANTS.

Statement of Financial Position as at 31 May 2015

	2015	2014
	$	$
Accumulated Funds	**$2,502,274**	$2,092,238
Represented by:		
Current Assets		
Cash at Bank	**414,008**	1,184,174
GST Refund Due	**1,785**	2,996
Prepayments	**6,708**	-
	422,501	1,187,170
Investments		
Term Deposits	**1,803,429**	652,870
NZ Fixed Interest	**6,000**	149,480
NZ Listed Property	**118,820**	105,274
NZ Equities	**10,349**	9,367
Australian Investments	**209,653**	171,045
American Investments	**-**	7,943
British Investments	**-**	1,071
	2,148,251	**1,097,050**
Total Assets	**2,570,752**	2,284,220
Current Liabilities		
Accounts Payable	**3,680**	15,916
Grants Payable	**44,798**	174,117
Related Party Payable	**-**	1,949
Donations Tagged Not Yet Allocated	**20,000**	**-**
	68,478	**191,982**
Net assets	**$2,502,274**	**$2,092,238**

Statement of Movements in Equity for the year ended 31 May 2015

	2015	2014
	$	$
Accumulated Funds		
Opening balance as at 1 June 2014	**2,092,238**	1,846,941
Plus: Net Surplus/(Deficit)	**410,036**	245,297
Closing Balance as at 31 May 2015	**$2,502,274**	$2,092,238

FINANCIAL STATEMENTS FOR THE YEAR ENDED 31 MAY 2015

THE FOLLOWING FINANCIAL STATEMENTS PROVIDE AN EXCERPT FROM THE AUDITED FINANCIAL STATEMENTS. THE UNQUALIFIED AUDIT REPORT WAS COMPLETED BY MARK CAMPBELL OF CAMPBELL AND CAMPBELL ACCOUNTING CONSULTANTS.

Statement of Financial Performance for the year ended 31 May 2015

	2015	2014
	$	$
Income		
Dividends	**16,380**	12,298
Donations & Fundraising– Appeal	**272,545**	283,256
Donations – Trust Waikato	**75,000**	65,000
Donations and Grants	**21,750**	144,209
Foreign exchange gain	**833**	-
Grants refunded	**5,878**	-
Interest – Appeal Funds	**38,917**	30,513
Interest – Investment Funds	**53,065**	48,821
Legacies Received	**10,488**	-
Realised gain on investments	**60**	-
Unrealised gain on investments	**25,649**	-
	520,565	584,097
Expenditure		
Accounting fees	**9,287**	8,107
Administration expenses	**20,975**	20,539
Advertising and promotion expenses	**4,006**	3,925
Audit fee	**4,550**	2,993
Foreign exchange loss	**-**	1,228
Fundraising expenses	**63,000**	90,300
Grants	**1,000**	188,117
Loss on realisation of investments	**218**	9,350
Portfolio management fees	**6,490**	5,338
Printing, stationery and postage	**1,003**	1,597
Unrealised loss on investments	**-**	7,306
	110,529	338,800
Net surplus/(deficit)	**$410,036**	$245,297

WAIKATO MEDICAL RESEARCH FOUNDATION HISTORY

In 1986 the Waikato Medical Research Foundation (Inc) was established and incorporated to promote, encourage and sustain medical research in the Waikato Region. At the time, Professor Michael Selby explains:
The aim was to undertake research that would be of benefit to the Waikato. Obviously we were hoping that the research would have wider applications than the Waikato. Inevitably, if you make any advances, the very nature of scientific work is that it gets published, and therefore you hope that the benefit will be widespread and therefore the people of the Waikato would benefit along with everybody else – that was the aim. So, we did put emphasis on publication, and therefore, of course quality – so that was part of the initial requirement.

The Waikato Medical Research Foundation has been established to enable ethical medical research to take place within the region. Medical Research will benefit everybody, and it warrants the support of all citizens.

In forming the Foundation, and going to the general public in the early years of fundraising, it stressed the importance that this is a local body. When initially formed, The foundation stressed to members of the general community in Hamilton and outlying areas that there were medical or health problems specific to the Waikato area, and that it was important to have a locally administered fund – and now 25 years on, the purposes of this Foundation are still as it was when initially formed.

The Trust was founded in 1986 with a capital pool of $1m.

"THE LEGACY OF THE PAST IS THE SEED THAT BRINGS FORTH THE PROSPERITY OF THE FUTURE"

Board of Trustees (1986)
Standing (left to right) Denis Jury, Andrea Donnison, Don Llewellyn, Ross McRobie
Sitting (left to right) Ken Mackay, John Gillies, Michael Selby, Brian Smith, James Grace

WAIKATO MEDICAL RESEARCH FOUNDATION
Discovery, Innovation, Progress.

Those who signed the Trust Deed in 1986 were:

Charles Beresford, Physician, Waikato Hospital,

John Gillies, Paediatrician, Waikato Hospital,

Jack Havill, Anaesthetist, Waikato Hospital,

Jim Grace, Solicitor,

Dryden Spring, a company director,

Michael Selby, Professor at the University of Waikato,

Jack Wilson, Head of TECH,

David Braithwaite, a company director,

Brian Smith, a chartered accountant and

Valerie O'Sullivan of Matamata.

TO OUR DONORS – THANK YOU

The Trustees of the Foundation wish to thank all who have generously donated since our inception in 1986. From 1986 to 2015, the Foundation has supported researchers in the following institutions:

Waikato District Health Board	$1,174,816
University of Waikato	$555,106
Faculty of Medical Health Sciences University of Auckland – Waikato Clinical School	$349,379
AgResearch	$589,307
Polytech	$41,310
Private researchers	$172,719
Totalling:	$2,882,637

Without your generous donations, the Foundation would not have been able to support 25+ years of research in the Waikato.

DONATION FORM

This form can be downloaded from our website: www.wmrf.org.nz
I wish to make a donation to the Waikato Medical Research Foundation

Please tick one :

O $50 O $100 O $200

O $500 O $1000 O $2000 Other (Amount $_____)

O I enclose a cheque made out to: Waikato Medical Research Foundation

O I have made a direct payment to WMRF Bank Account: Westpac 030 306 0208170 01
(Please include your name as reference for the payment)

O Please send me a receipt

As we are registered with the Charities Commission (Charities Commission No: CC20443), all donations to Waikato Medical Research Foundation over $5.00 are tax rebatable.
Please complete your details and post / fax for a receipt.

Name of donor: _____

Address: _____

Daytime telephone: _____

For future contact, we would like to e-mail interested parties, and if you wish to receive information from us, please complete below:
Email address: _____

WAIKATO MEDICAL RESEARCH FOUNDATION
Discovery, Innovation, Progress.

Post to: Private Bag 3200, Waikato Mail Centre Hamilton 3240
Phone: (07) 839 8750 **Fax:** (07) 839 8712
Email: wmrf@waikatodhb.health.nz
Web: www.wmrf.org.nz

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