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The Heart Research Institute
Projects 2015 • 2016

The Heart Research Institute
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HEART RESEARCH INSTITUTE
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APPLIED MATERIALS GROUP
DEVELOPING NEXT-GENERATION VASCULAR BIOMATERIALS

Summary

This project will systematically develop proactive biocompatible surfaces integrated onto metallic cardiovascular devices including coronary stents and artificial heart valves. It includes: 1) plasma physics and vascular bioengineering; 2) cellular and haemocompatibility studies and 3) *in vivo* pre-clinical testing.

Background

Metallic cardiovascular implants, such as stents, used in the treatment of heart disease are not compatible with blood. They cause inflammation at the site of implantation and increase the risk of blood clots forming. We have developed a unique method of binding bioactive protein layers to the surface of metal alloys, and shown a significant improvement in their compatibility. Stents coated using our technology stand to dramatically improve the treatment of cardiovascular disease. The project will closely integrate vascular biology with bioengineering and physics to enable development of implantable materials for treatment of cardiovascular diseases that feature proactive biocompatibility through biomimicry.

Overview of Studies

These studies will investigate a number of newly developed biomaterials for application in endovascular medical devices in a cutting edge translational research program. There may be unique opportunities to integrate basic science discoveries with clinical studies to gain novel insights into cardiovascular biology and disease. The multi-disciplinary nature of the project will provide exposure to physics, biochemistry and vascular biology, interacting with a diverse research team. In vitro assays established at the HRI will assess blood compatibility, endothelial and smooth muscle cell interactions in conjunction with the physical and chemical properties of our unique surfaces. Evaluation of our most promising devices is currently underway in established pre-clinical small and large animal models.

For more information contact: Dr Steve Wise, steve.wise@hri.org.au

CELL THERAPEUTICS GROUP
CARDIOVASCULAR REGENERATION USING INDUCED PLURIPOTENT STEM CELLS

Summary

Induced pluripotent stem cells (iPSCs) are a novel stem cell type that holds great promise in augmenting cardiovascular regeneration.

Background

In 2006, Yamanaka et al reported that mouse fibroblasts could be reprogrammed into induced pluripotent stem (iPS) cells by viral transduction of the four transcription factors; Oct3/4, Sox2, Klf4 and c-Myc. This groundbreaking work represents an exciting development in regenerative medicine, as these iPS cells not only overcome the ethical concerns related to the use of human eggs or earlier embryos for deriving stem cells, but also have the potential to produce patient-specific stem cells.

However, currently all directed reprogramming of differentiated cells have relied on gene delivery through viral vectors that integrate into the cell's genome. Such cells will not be useful as therapeutic reagents. Therefore, this project is intended to support the goals to derive and characterise iPS cells using a non-viral approach (mRNA based approach); to differentiate the iPS cells into vascular progenitors; and to assess their therapeutic efficacy to (1) improve perfusion in pre-clinical models of limb ischaemia, (2) their ability to augment tissue healing and (3) their ability to incorporate into hydrogel matrices which could be used ultimately as a source of new functional myocardium, skin grafts or vascular conduits. Dr Patel heads the Cell Therapeutics Group at the Heart Research Institute, which has considerable experience with the generation of iPSCs via viral methods in collaboration with Prof John Cooke's group at Stanford University and Houston Methodist Hospital. Similarly, this group has recently generated iPSCs via modified mRNA transfection, and tested their angiogenic potential *in vivo.*

Overview of Studies

Differentiation of iPSCs to iPSC-ECs: The reprogrammed cells will be characterised *in vitro* to confirm their pluripotentiality. To initiate differentiation, the iPS cells will be cultured in non-adhesive dishes to form embryoid body aggregates that can spontaneously differentiate.

The cells are then dissociated and purified for the EC-specific markers vascular endothelial (VE)-cadherin and PECAM-1 by FACS. The EC phenotype of the iPS-derived ECs is confirmed by gene expression assays. Our group also has experience in generating an EC-specific reporter construct for purification and bioluminescence tracking of ECs. These novel techniques therefore allow for dynamic tracking of ECs *in vitro* and *in vivo*.

Assessment of the function of iPS-derived ECs in vitro: Established methods will be employed to characterise the function of these cells, including assays for cell proliferation, cell migration, and tube formation in matrigel. We will further assess incorporation of iPS-ECs into biosynthetic hydrogels to form functional vascularised tissue.

In vivo assessment of iPS-EC function: The mouse hindlimb ischaemia model has been well characterised in our laboratory, and the effects of these cells in promoting expansion of the microvasculature and tissue perfusion will be assessed. Moreover, the ability of iPSC-ECs to promote wound healing in a mouse tissue injury model will be evaluated.

For more information contact: Dr Sanjay Patel, sanjay.patel@hri.org.au

FREE RADICAL GROUP

WHAT ARE THE KEY MECHANISMS INVOLVED IN HYDROXYL RADICAL-INDUCED PROTEIN DAMAGE?

Summary

This project will investigate the mechanisms and products formed during hydroxyl radical-induced damage to amino acids, peptides and proteins.

Background

Free radicals are unavoidable by-products of aerobic metabolism, and are also generated by exposure to external agents (e.g. smoke, radiation, asbestos, drugs). Hydroxyl radicals (HO·) are particularly reactive radicals that are generated in biological systems and proteins are major targets for these species. Thus, elevated levels of HO··specific protein oxidation products are found in various diseases including atherosclerosis (hardening of the arteries) and cataracts. HO· damage to proteins is also relevant in numerous other situations (e.g. plant stress, food spoilage, sterilisation of foods/pharmaceuticals using radiation).

Despite the widespread nature and applicability of these reactions, detailed mechanistic information for HO··mediated damage to proteins, and the exact products formed are poorly defined under physiologically relevant conditions. Gaining greater understanding of these processes is important, as some of the oxidation products (e.g. hydroperoxides) are key intermediates that can induce chain reactions and exacerbate damage.

Overview of Studies

This project will identify which amino acids in peptides and proteins are most susceptible to HO··induced oxidation in the presence of oxygen, including determining the products formed, the relative proportions of each product, and the mechanisms of peptide and protein fragmentation. Initial studies will involve the study of relatively simple peptides, and these results will guide investigations in more complex peptides/model proteins as well as proteins that are relevant to the development of human disease. The data will also enable the potential development of novel biomarkers for evaluating the contribution of HO··induced damage in disease processes. The use of novel antioxidants to prevent or reverse these reactions will also be evaluated.

The project will utilise a wide variety of methods, including (but not limited to) UV/Vis spectroscopy, EPR spectroscopy, HPLC/UPLC and LC/MS. This project would suit someone with a chemistry/biochemistry background with a strong interest in analytical and protein chemistry.

Key references

1 Davies, M. J. (2005) The oxidative environment and protein damage. *Biochim. Biophys. Acta,* 1703, 93–109.

2 Headlam, H. A., Gracanin, M., Rodgers, K. J., Davies, M. J. (2006) Inhibition of cathepsins and related proteases by amino acid, peptide, and protein hydroperoxides, *Free Radic. Biol. Med.,* 40, 1539–1548.

3 Morgan, P. E., Pattison, D. I., Davies, M. J. (2012) Quantification of hydroxyl radical-derived oxidation products in peptides containing glycine, alanine, valine, and proline, *Free Radic. Biol. Med.,* 52, 328-339.

4 Morgan, P. E., Pattison, D. I., Hawkins, C. L., Davies, M. J. (2008) Separation, detection, and quantification of hydroperoxides formed at side-chain and backbone sites on amino acids, peptides, and proteins, *Free Radic. Biol. Med.,* 45, 1279–1289.

For more information contact: Dr David Pattison, david.pattison@hri.org.au

FREE RADICAL GROUP

ASSESSING THE NATURE OF PROTEIN DAMAGE INDUCED BY PEROXYL RADICALS AND ITS BIOLOGICAL CONSEQUENCES

Summary

This project will investigate the mechanisms and products formed during peroxyl radical-induced damage to amino acids, peptides and proteins.

Background

Free radicals are generated in biological systems as by-products of normal cellular redox processes, or via the interaction of cells and tissues with a number of external agents including radiation. It is well established that amino acids, peptides and proteins are major targets for radicals, with protein oxidation occurring during the normal aging process, and in many human diseases (e.g. in cataracts, heart disease). Free-radical mediated damage to proteins is also relevant to the food, agricultural and pharmaceutical industries, as properties including solubility, hydrophobicity, conformation, enzyme activity and digestibility are affected. There is considerable evidence that hydroperoxides (ROOH) are key reactive intermediates produced during radical damage to proteins.

Decomposition of hydroperoxides can yield secondary radicals (e.g. peroxyl (ROO˙), alkoxyl (RO˙)) that can exacerbate damage by reacting with other targets (e.g. amino acid/protein residues, DNA bases) and can lead to protein-protein and protein-DNA cross-link formation. The generation of diTyr crosslinks is well characterised in these reactions, but recent data (Arenas *et al,* 2013) indicate that alternative mechanisms and poorly defined products are also involved in these processes.

Overview of Studies

This project will utilise a range of peroxyl radical generating systems to examine the oxidation of peptides and proteins in detail, with the aim of identifying novel products and quantifying their abundance relative to established products. Initial studies will involve the study of simple model peptides, which will guide studies on more complex peptides and model proteins.

These data will allow the potential biological relevance and consequences of peroxyl radical reactions to be more completely understood. In addition, the efficacy of novel antioxidants in preventing or reversing this damage will also be evaluated.

This project will use a wide variety of analytical chemical methods, including UV/Vis spectroscopy, fast reaction kinetics (stopped flow), EPR spectroscopy, HPLC/UPLC and LC/MS, as well as biochemical approaches such as cell culture, gel electrophoresis and Western blotting. This project would suit someone with a chemistry/biochemistry background with a strong interest in analytical and protein chemistry.

Key references

1 Davies, M. J. (2005) The oxidative environment and protein damage. *Biochim. Biophys. Acta,* 1703, 93–109.

2 Arenas, A., Lopez-Alarcon, C., Kogan, M., Lissi, E., Davies, M. J., Silva, E. (2013) Chemical modification of lysozyme, glucose 6-phosphate dehydrogenase, and bovine eye lens proteins induced by peroxyl radicals: Role of oxidizable amino acid residues. *Chem. Res. Toxicol.,* 26, 67-77.

3 Morgan, P. E., Pattison, D. I., Hawkins, C. L., Davies, M. J. (2008) Separation, detection, and quantification of hydroperoxides formed at side-chain and backbone sites on amino acids, peptides, and proteins, *Free Radic. Biol. Med.,* 45, 1279–1289.

4 Gracanin, M., Hawkins, C. L., Pattison, D. I., Davies, M. J., (2009) Singlet-oxygen-mediated amino acid and protein oxidation: Formation of tryptophan peroxides and decomposition products, *Free Radic. Biol. Med.,* 47, 92-102.

For more information contact: Dr David Pattison, david.pattison@hri.org.au

HIGH BLOOD PRESSURE GROUP
ROLE OF NEUROTRANSMITTERS IN REGULATING SYMPATHETIC NERVE ACTIVITY

Summary

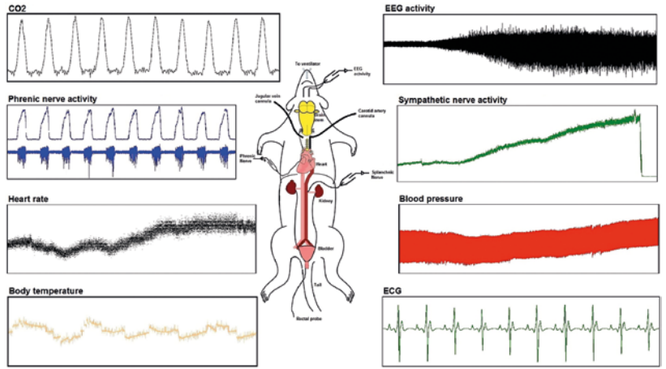
In the High Blood Pressure Group we investigate the way that different neurons in the brainstem and spinal cord control the heart, blood vessels and breathing, and the way that these pathways learn and remember information (neuroplasticity). From a disease perspective, we study abnormalities of these critical neural pathways that can lead to high blood pressure and other health problems.

Project areas:

1. Role of neurotransmitters in the brainstem and spinal cord in regulating sympathetic nerve activity, the heart, blood vessels and adrenal medullary hormone release.

- **PACAP** and other peptides found in cardiorespiratory nuclei in the spinal cord and brainstem: these peptides raise or lower blood pressure and heart rate and can modify the way the brain responds to particular stimuli, such as pain or low oxygen levels.
- **Orexin** and other hypothalamic peptides: Peptides originating from this region of the brain are involved in many autonomic functions including fluid-balance, blood pressure control, feeding and sleeping; activities that involve coordination with cardiorespiratory function.
- **Serotonin** and other raphe neurotransmitters: An area of the brainstem that controls temperature regulation as well as sensing carbon dioxide levels and altering blood pressure.

2. Role of central neurotransmitters in disorders characterised by prolonged increases in sympathetic nerve activity



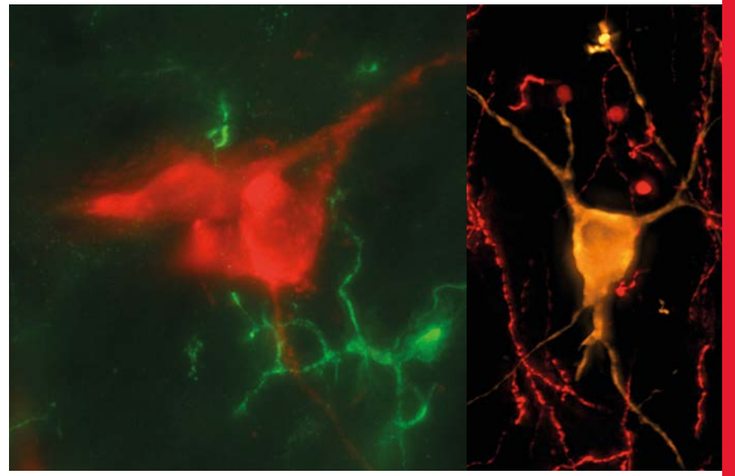
- **Obstructive Sleep Apnoea (OSA):** characterised by intermittent hypoxia, chemoreceptor activation, sympathetic activation, and hypertension.
- **Epilepsy:** resulting in prolonged sympathetic activation and a 20-30% risk of sudden death following *grand mal* seizure due to cardiac arrhythmia.
- **Heart Failure:** leading to a vicious cycle of low cardiac output, hypotension, sympathoactivation and further target organ damage to the heart.

For more information contact: Professor Paul Pilowsky, paul.pilowsky@hri.org.au

HIGH BLOOD PRESSURE GROUP
ROLE OF NEUROTRANSMITTERS IN REGULATING SYMPATHETIC NERVE ACTIVITY

Techniques used include:

- Whole animal *in vivo* physiology and pharmacology experiments (rats and mice),
  - o Including induction and monitoring of anaesthesia, ECG, blood pressure and nerve activities, evoking homeostatic reflexes (eg. baroreflex, chemoreflex and somatosympathetic reflex), and central administration of neurotransmitter agonists or their antagonists.
  - o Intracellular and extracellular electrophysiology.
- Combined *in situ* hybridisation and fluorescence immunohistochemistry,
  - o Includes perfusion, tissue harvest and preparation, the combined ISH/IHC protocol and slide preparation.
- Molecular biology
  - o Including quantitative real-time PCR, vector construction and transfection,
  - o Optogenetics and selective gene-targeted cell destruction
- Microscopy
  - o Light, fluorescence and confocal



Neurons (red, orange) and microglia (brain macrophages; green) in cardiorespiratory regions of the brainstem.

For more information contact: Professor Paul Pilowsky, paul.pilowsky@hri.org.au

IMMUNOBIOLOGY GROUP
‘MICRO’MANAGING ANGIOGENESIS IN DIABETES

Background

MicroRNAs (miRNAs) are short non-coding RNAs that function as negative post-transcriptional regulators of gene expression. miRNAs bind to complementary sequences on target messenger RNA transcripts (mRNAs), usually resulting in translational repression or gene silencing. Increasing evidence demonstrates that post-transcriptional regulation by miRNAs is critically important in many aspects of development, homeostasis and disease. Angiogenesis is the fundamental process in which new blood vessels develop from existing vessels in response to external signals detected by vascular endothelial cells. miRNAs have an emerging role in the modulation of physiological hypoxia-induced angiogenesis and pathological inflammatory-driven angiogenesis. Our laboratory has recently identified in preliminary experiments novel miRNAs that regulate angiogenesis. Their role in various angiogenic disease and their mechanisms of action have not as yet been elucidated.

Overview of Studies

This project will explore the role of our miRNAs of interest *in vitro* and *in vivo*. Using an adenoviral approach, we aim to investigate their regulation following overexpression of the hypoxia inducible factor-1α (HIF-1α) and nuclear factor-κB (NF-κB) pathways, two key transcription factors that drive hypoxia and inflammation-induced angiogenesis, respectively. Furthermore, the *in vitro* angiogenic function of our miRNAs of interest will be investigated using both Pre-miR miRNA Precursor Molecules and Anti-miR miRNA inhibitors, which increase or decrease specific miRNA expression. These studies will be extended *in vivo* using the hindlimb model of ischemia-driven angiogenesis and the femoral artery cuff model of inflammation-induced angiogenesis. This project will provide the opportunity to learn a broad range of techniques including cell culture, RT-PCR and western blotting, gene transfer, functional assays to test *in vitro* angiogenesis and animal models of angiogenesis. All the methods and equipment that are required for this project are present at the Heart Research Institute.

For more information contact: Dr Christina Bursill, christina.bursill@hri.org.au

IMMUNOBIOLOGY GROUP
EFFECT OF HIGH-DENSITY LIPOPROTEINS ON EARLY-STAGE VS LATE-STAGE ATHEROSCLEROSIS

Summary

High-Density Lipoproteins (HDL) are thought to be protective against the development of atherosclerosis. This project seeks to determine whether HDL has different effects in early-stage versus late-stage atherosclerosis.

Background

There is overwhelming epidemiological evidence demonstrating that High-Density Lipoproteins (HDL) have anti-atherosclerotic properties. For example, it is shown that the risk for myocardial infarction increases by about 25% for every 0.13 mmol/L decrement in serum HDL below median values, while others have reported an association between low serum HDL and susceptibility to plaque rupture.

Infusion of HDL has also been shown to have plaque regressing and stabilising effects in animal models where infusions of HDL reduce atherosclerotic plaque size by 35-55%, along with a marked reduction in plaque inflammation - a key determinant of plaque stability.

Most people who present to clinic with cardiovascular problems are most likely to have atherosclerotic plaques that are at an advanced stage, however most preclinical models investigating the effects of HDL on atherosclerosis and the underlying mechanisms look at early-stage lesions. HDL exert their beneficial effects by effluxing cholesterol from atherosclerotic plaques and transporting the cholesterol back to the liver for processing. HDL also have potent anti-inflammatory properties.

As there are distinct phenotypic differences between advanced and early-stage plaques, as well as differences in immunological and inflammatory processes, it raises the possibility that HDL also has different effects at different stages of atherosclerosis.

Overview of Studies

This project will investigate the effect of HDL on advanced versus early-stage atherosclerotic plaques and assess differences in the mechanisms of action. This project will provide the opportunity to learn about animal models of atherosclerosis. Other skills will include immunohistochemistry, ELISAs, RT-PCR, Western Blotting as well as microCT for 3D analysis of atherosclerotic plaques.

For more information contact: Dr Christina Bursill, christina.bursill@hri.org.au

IMMUNOBIOLOGY / HIGH BLOOD PRESSURE GROUPS

THE ROLE OF INFLAMMATORY MONOCYTES IN THE CENTRAL NERVOUS SYSTEM IN HYPERTENSION

Labs/Investigators: Christina Bursill, Melissa Farnham, Paul Pilowsky and Stacy Robertson

The Immunobiology and High Blood Pressure Groups are concerned with identifying cell signalling pathways and cell types in the vasculature, and central nervous system that are important in the genesis of cardiovascular disease.

The role of inflammatory monocytes in the central nervous system in hypertension

Chemokines are small proteins that direct the migration of inflammatory cells, including monocytes and microglia, to sites of injury or repair. There is increasing evidence that activated monocytes, which express a specific set of chemokine receptors, are responsible for inflammation in regions of the brainstem responsible for regulating blood pressure. We aim to investigate the role of chemokines, which recruit circulating monocytes and activate microglia, in the development of hypertension.

To achieve this we will:

- Examine the translocation of circulating monocytes into the brainstem
- Determine if translocated monocytes can transform into microglia or activate microglia
- Determine the extent to which local microglia or transformed monocytes can cause a prolonged stimulation of brain nuclei that control the circulation resulting in changes in blood pressure.
- Determine the inflammatory status of circulating monocytes isolated from rats at different stages of hypertension and determine if chemokines are present in critical areas of the brainstem.

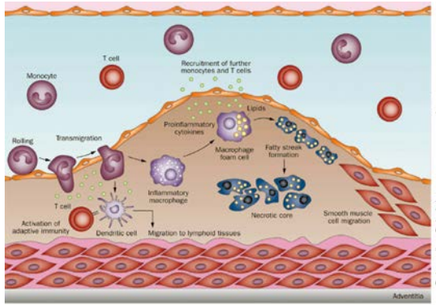
Technical approaches may include: quantitative RT-PCR, multiple label immunohistochemistry, flow cytometry and animal models of hypertension.

All methods/techniques are well established in our laboratory.

All training will be provided, and experiments conducted, in our modern facility in the heart of Newtown.

For more information contact: Dr Christina Bursill, christina.bursill@hri.org.au or Professor Paul Pilowsky, paul.pilowsky@hri.org.au

INFLAMMATION GROUP
ROLE OF LDL MODIFICATION IN CELLULAR DYSFUNCTION IN ATHEROSCLEROSIS



Summary

This project will examine how the oxidation of low-density lipoprotein (“bad” cholesterol) under inflammatory conditions induces cellular dysfunction in the artery wall and will investigate novel strategies to modulate these damaging reactions.

Background

Atherosclerosis is characterised by the accumulation of lipids within cells in the intima of the artery wall, which progresses over decades to form the complex lesions responsible for the clinical symptoms: plaque rupture, clot formation and blockage of blood flow and heart attack or stroke. Globally, atherosclerosis causes ~40% of all deaths in developed countries. The number of deaths is increasing because of the epidemic of obesity, diabetes and associated metabolic disorders, which cause adverse changes to plasma lipid profiles.

A key event in the initiation of atherosclerosis is the oxidative modification of low-density lipoprotein (oxLDL), which causes the uncontrolled cellular uptake of lipid and triggers a cascade of inflammatory events (image taken from Heine, G. H. *et al.* (2012) Monocyte subpopulations and cardiovascular risk in chronic kidney disease; *Nat. Rev. Nephrol.* doi:10.1038/nrneph.2012.41)

Human lesions contain significant amounts of LDL modified by myeloperoxidase (MPO), an enzyme released under inflammatory conditions that produces potent chemical oxidants. MPO is a major risk factor for the development of coronary artery disease and a powerful prognostic agent for predicting the outcome of patients with cardiac symptom.

Although MPO-modified LDL is highly relevant to human disease, there are a lack of data relating to the mechanisms involved in the cellular processing and accumulation of MPO-modified LDL in human cells and the resulting detrimental consequences, which will be examined in this project.

Project Overview

In this project, we will examine the role of different types of modified LDL on macrophage cell function using cultured primary human cells.

LDL will be exposed to hypochlorous acid (HOCl) or hypothiocyanous acid (HOSCN), which are the major MPO-derived oxidants formed *in vivo*. We will examine the extent of uptake and turnover of each type of modified LDL in different cell types, and the resulting consequences on cellular behaviour and macrophage polarisation using a variety of different functional assays, together with examining changes in protein and gene expression.

The project will provide training and experience with cultured primary cells and will utilise a range of laboratory techniques including Western blotting, ELISA, real-time RT-PCR, flow cytometry, HPLC and EPR spectroscopy.

For more information contact: Associate Prof Clare Hawkins, clare.hawkins@hri.org.au

INFLAMMATION GROUP
CHLORINATED NUCLEOSIDES - NOVEL MEDIATORS OR BIOMARKERS OF DISEASE?

Summary

This project will examine how chlorination of RNA and DNA nucleosides modulates the function of vascular cells and whether this contributes to inflammatory disease and the development of atherosclerosis.

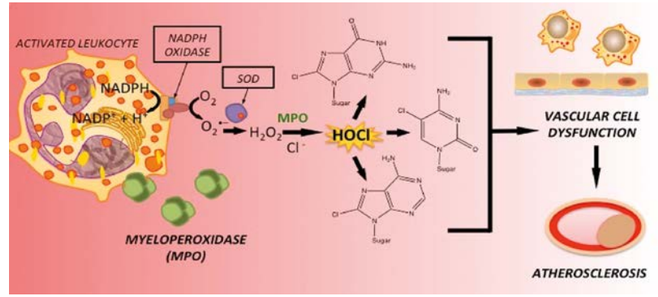
Background

Chlorine is a potent disinfectant, which is mainly present in aqueous solution as hypochlorous acid (HOCl), and molecular chlorine (Cl_2).

Hypochlorous acid is used widely to kill harmful bacteria in both drinking water and swimming pools. This disinfectant is also produced in the body by activated leukocytes (white blood cells) via the myeloperoxidase-catalysed reaction of hydrogen peroxide (H_2O_2) with chloride (Cl^-) ions.

The chemistry of HOCl in biological systems has attracted considerable attention, as excessive or misplaced production of this chemical during prolonged inflammation, where there is an infiltration of leukocytes, damages tissue and causes disease. HOCl chlorinates the nucleoside building blocks of RNA and DNA, and these products are seen in diseased tissue.

Currently it is not known whether RNA / DNA chlorination is a consequence of the disease process, or whether these modified nucleosides play a role in disease pathology.



Overview of Studies

This project will build on our novel data showing that chlorinated nucleosides perturb the expression of key stress and toxicity genes, which has a significant impact on cellular function. These cellular changes are detrimental to function, and may play a role in disease development. This has important toxicological significance for chlorinated drinking water supplies, in addition to providing novel insights into inflammation-induced disease. We will use a mass spectrometry approach to assess the extent and rate of uptake and turnover of chlorinated nucleosides by different human vascular cells and a molecular biology approach to define the consequences of chlorinated nucleosides on vascular cell function. This project will provide training in primary cell culture, analytical techniques including HPLC and LC-MS, together with real-time RT-PCR (gene expression), Western blotting and ELISA (protein expression) and flow cytometry (cell function / death).

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INFLAMMATION GROUP

INVESTIGATING THE MOLECULAR MECHANISMS INVOLVED IN VASCULAR CELL DAMAGE AND DEATH, WITHIN THE SETTING OF ATHEROSCLEROSIS

Summary

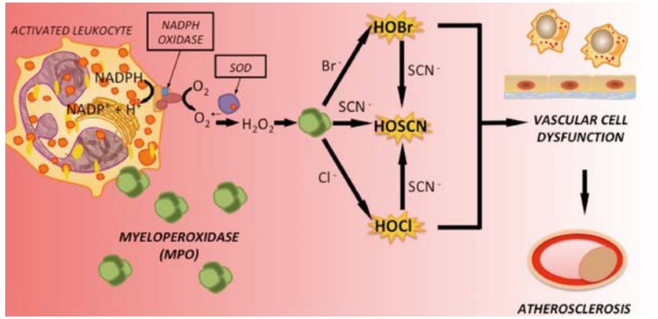
This project will investigate how inflammatory oxidants modulate the cellular redox environment and cause changes in gene expression, which may contribute to the development of disease.

Background

There is epidemiological, clinical, and experimental evidence that cellular stress and excessive inflammation are causally linked to various pathological conditions including atherosclerosis (hardening of the arteries). Macrophage infiltration and the resultant oxidant formation within atherosclerotic lesions in the vascular wall leads to oxidative stress, damage and ultimately death to cells of the vasculature. This accelerates lesion formation and can also result in the destabilisation of lesions, which ultimately triggers thrombosis and heart attacks. This project will focus on delineating the precise intracellular mechanisms and pathways that result from exposure to neutrophil and macrophage-derived oxidants to better inform the development of novel therapies for atherosclerosis.

Overview of Studies

This project will examine the pathways involved in vascular cell damage, with a focus on understanding how inflammatory oxidants modulate apoptosis, the oxidative stress response, and transcriptional regulation in various vascular cell types. Techniques that will be employed to achieve this goal include using endothelial, vascular smooth muscle and macrophage cell culture models, gene analysis

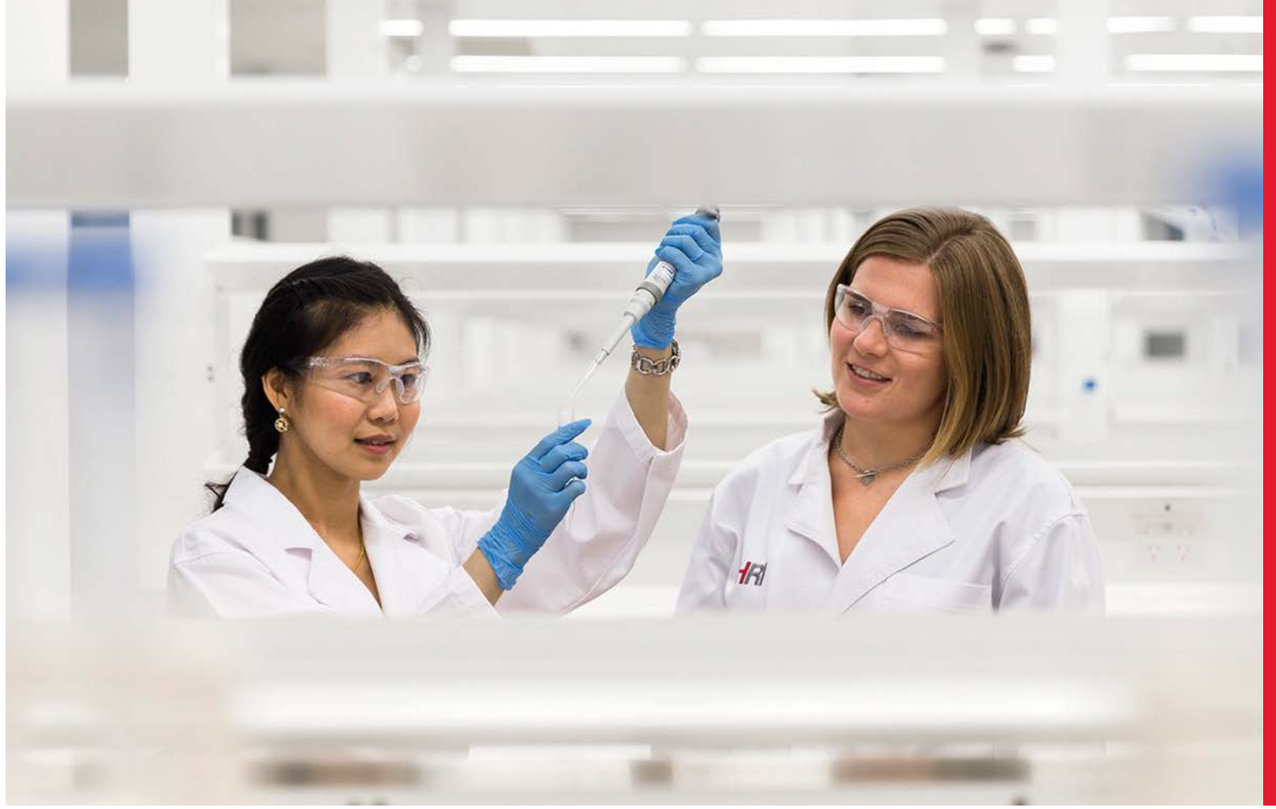


by quantitative real-time PCR, protein expression analysis by Western blotting, and flow cytometry to analyse cellular dysfunction and death. The detailed knowledge relating to the biochemical mechanisms of vascular cell damage during inflammation is important for the design of new therapeutic agents to modulate inflammation and slow the progression of atherosclerosis.

Relevant Publications from our Group

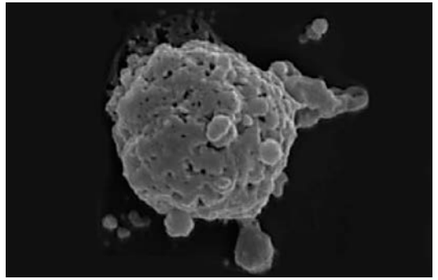
- Rayner *et al.,* Comparative reactivity of myeloperoxidase-derived oxidants with mammalian cells. *Free Radic. Biol. Med.* 71: 240-255; 2014.
- Lloyd *et al.,* Comparative reactivity of the myeloperoxidase-derived oxidants hypochlorous acid and hypothiocyanous acid with human coronary artery endothelial cells. *Free Radic. Biol. Med.* 65: 1352-1362; 2013.
- Barrett, T. J.; Hawkins, C. L. Hypothiocyanous acid: Benign or deadly? *Chem. Res. Toxicol.* 25: 263-273; 2012.

For more information contact: Associate Prof Clare Hawkins, clare.hawkins@hri.org.au



THROMBOSIS GROUP

INVESTIGATING THE ROLE OF CELL DEATH PATHWAYS IN REGULATING THE PROINFLAMMATORY FUNCTION OF PLATELETS AND LEUKOCYTES DURING ISCHAEMIA-REPERFUSION INJURY



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This project will examine how the oxidation of low-density lipoprotein ("bad" cholesterol) under inflammatory conditions induces cellular dysfunction in the artery wall and will investigate novel strategies to modulate these damaging reactions.

Background

Atherosclerosis is characterised by the accumulation of lipids within cells in the intima of the artery wall, which progresses over decades to form the complex lesions responsible for the clinical symptoms: plaque rupture, clot formation and blockage of blood flow and heart attack or stroke. Globally, atherosclerosis causes ~40% of all deaths in developed countries.

The number of deaths is increasing because of the epidemic of obesity, diabetes and associated metabolic disorders, which cause adverse changes to plasma lipid profiles.

A key event in the initiation of atherosclerosis is the oxidative modification of low-density lipoprotein (oxLDL), which causes the uncontrolled cellular uptake of lipid and triggers a cascade of inflammatory events (image taken from Heine, G. H. *et al.* (2012) Monocyte subpopulations and cardiovascular risk in chronic kidney disease; *Nat. Rev. Nephrol.* doi:10.1038/nrneph.2012.41)

Human lesions contain significant amounts of LDL modified by myeloperoxidase (MPO), an enzyme released under inflammatory conditions that produces potent chemical oxidants. MPO is a major risk factor for the development of coronary artery disease and a powerful prognostic agent for predicting the outcome of patients with cardiac symptom. Although MPO-modified LDL is highly relevant to human disease, there are a lack of data relating to the mechanisms involved in the cellular processing and accumulation of MPO-modified LDL in human cells and the resulting detrimental consequences, which will be examined in this project.

Project Overview

In this project, we will examine the role of different types of modified LDL on macrophage cell function using cultured primary human cells. LDL will be exposed to hypochlorous acid (HOCl) or hypothiocyanous acid (HOSCN), which are the major MPO-derived oxidants formed *in vivo*. We will examine the extent of uptake and turnover of each type of modified LDL in different cell types, and the resulting consequences on cellular behaviour and macrophage polarisation using a variety of different functional assays, together with examining changes in protein and gene expression.

The project will provide training and experience with cultured primary cells and will utilise a range of laboratory techniques including Western blotting, ELISA, real-time RT-PCR, flow cytometry, HPLC and EPR spectroscopy.

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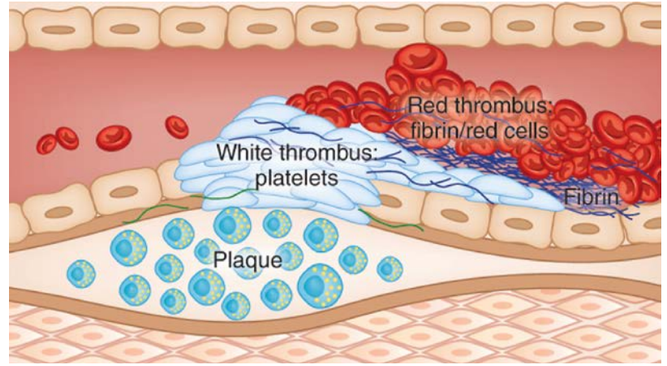
THROMBOSIS GROUP

CHLORINATED NUCLEOSIDES - NOVEL MEDIATORS OR BIOMARKERS OF DISEASE?

Atherothrombosis is a major healthcare problem that affects >40% of the adult population. In particular, the development of arterial thrombosis in the coronary or cerebral circulation (causing acute myocardial infarction and ischaemic stroke, respectively) is responsible for more deaths in the community than any other disease process. Despite intense investigation over the last 40 years into the discovery and development of more effective anti-platelet drugs, the impact of these therapies on mortality rates has remained disappointingly low, with less than 1 and 6 patients taking anti-platelet therapies avoiding a fatal thrombotic event. This situation is likely to worsen in the future due to the rapidly growing incidence of obesity, diabetes and the metabolic syndrome. These diseases are typically more resistant to the benefits of anti-platelet therapy, thus there is a pressing need for the identification and development of more effective approaches.

Our laboratory has recently defined a new pathway promoting platelet aggregation and thrombus development that involves biomechanical platelet activation. More recently, we have identified that this pathway is dysregulated in diabetes and leads to enhanced platelet-endothelial interaction through a molecular process that is linked to atherogenesis. In this project we aim to identify the molecular mechanisms by which hyperglycemia leads to enhanced biomechanical platelet activation, and the relevance of this pathway to platelet-endothelial and platelet-platelet adhesive interactions linked to atherothrombosis. This project involves the study of platelet function from genetically-manipulated mouse models of diabetes as well as patients with Type I and II diabetes. The role of platelet scavenger receptors, including CD36 and SR-BI, receptors for advanced glycation end-products (AGEs) and key components of the oxidative stress



pathways in platelets will be examined for their ability to promote biomechanical platelet activation. This project utilises a broad range of techniques including detailed cell biology and signalling assays, *in vitro* perfusion assays, flow cytometry, confocal microscopy and *in vivo* models of endothelial dysfunction and thrombosis.

Relevant publications from our group:

- Jackson SP. *Nature Med.* 17(11):1423-1436, 2011
- Jackson SP and Schoenwaelder SM. *Nature Reviews Drug Discovery,* 2:775-789, 2003
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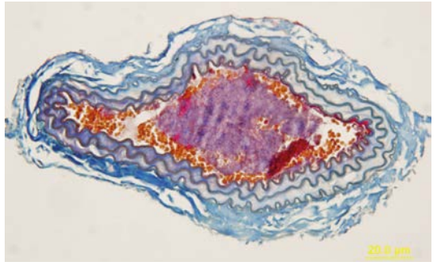
THROMBOSIS GROUP
IDENTIFYING NOVEL APPROACHES TO FACILITATE BLOOD CLOT DISSOLUTION



Blood platelets play a critical in the development of occlusive arterial blood clots (thrombi), precipitating diseases such as heart attack and ischaemic stroke. The rapid reperfusion of occluded blood vessels to minimise tissue death is a key treatment goal in patients suffering heart attack and stroke, with the administration of thrombolytic therapy an important means of establishing reperfusion. This is usually achieved through administration of fibrinolytic agents modelled on tissue-type plasminogen activator (tPA). However, thrombolytic therapy is not without its limitations, with lysis resistant blood clots, as well as hemorrhage presenting as major complications.

One of the main factors delaying reperfusion and increasing the risk of reocclusion of cerebral vessels is the presence of platelets in arterial thrombi. Platelets inhibit thrombolysis through multiple mechanisms and numerous preclinical and clinical studies have demonstrated the benefits of adjunctive anti-platelet therapy to enhance cerebral reperfusion and reduce reocclusion following thrombolysis. Unfortunately in stroke patients, the benefits of combined antiplatelet and thrombolytic therapy are partially offset by the increased risk of life-threatening intracerebral bleeding, limiting the widespread use of this approach.

Our laboratory has recently demonstrated that inhibitors of PI 3-kinase (PI3Kβ), when adminsitered alone or with tPA, are highly effective at promoting thrombus dissolution, without markedly increasing tail bleeding times. These results raise the possibility that PI3Kβ inhibitors may represent a safe and effective adjuvant therapy for the treatment of stroke. This project will examine the potential use of PI3Kβ inhibitors as adjuvant therapy for stroke and compare their safety and efficacy with that of currently used anti-platelet agents. Studies will involve the use of *in vivo* models of thrombosis and thrombolysis, *in vitro* flow-based assays, genetic mouse models and state-of-the-art imaging systems (confocal microscopy, intravital microscopy), complemented with *in vitro* analysis of platelet function. These studies will not only provide important insight into our understanding of blood clot formation, but may also lead to new approaches to regulate the size and stability of blood clots forming in the body, providing major clinical benefit in the delivery of thrombolytic therapy (blood clot removal).

Relevant publications from our group:

- Jackson SP and Schoenwaelder SM. *Nature Reviews Drug Discovery,* 2:775-789, 2003
- Jackson SP, Schoenwaelder SM, et al, *Nature Medicine,* 11(5):507-514, 2005.
- Jackson SP and Schoenwaelder SM. *Cell Mol Life Sci,* 63(10):1085-90, 2006.
- Schoenwaelder SM, et al, *J Biol Chem,* 282(39):28648-58, 2007.
- Jackson SP and Schoenwaelder SM. *Curr Top Microbiol Immunol,* 346:203-24, 2010.
- Schoenwaelder SM, et al, *J Biol Chem,* 285(4):2886-2896, 2010.
- Jackson SP. *Nature Medicine.* 17(11):1423-1436, 2011

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TRANSLATIONAL AND BIOENGINEERING GROUP
TRANSLATING RESEARCH FROM THE LAB BENCH TO THE CLINIC

Summary

In the Translational Research Group we investigate the mechanisms of angiogenesis, cardiovascular complications in diabetes, androgen and age-related vascular regeneration and repairs. Our group aims to develop and apply therapeutic angiogenesis either through the facilitation of vessel growth or through the delivery of progenitor cells. These new methods hold tremendous potential as new treatments for cardiovascular disease.

Current Projects

1. **The Role of Thioredoxin Interacting Protein (TXNIP) in the Pathogenesis of Diabetic Vascular Complications**

   Endothelial damage, impaired endothelial regeneration and endothelial dysfunction play a critical role in the onset and progression of diabetic vascular complications. Chronic hyperglycemia is a major initiator of diabetic vascular complications. TXNIP, an exquisitely glucose-inducible gene, is a multi-functional protein that is emerging as a key regulator of endothelial biology.

   This project seeks to investigate the role of TXNIP in the pathogenesis of diabetic vascular complications, with a particular focus on the mechanisms by which TXNIP modulates diabetes-related susceptibility to endothelial damage and dysfunction.

2. **The protective effects of fenofibrate in diabetes-related susceptibility to ischaemia**

   The vascular complications of diabetes are associated with impaired angiogenesis in response to ischaemia and impaired tolerance to hypoxia, though the mechanisms for this are poorly understood. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) placebo-controlled randomised trial, led by our collaborator Professor Anthony Keech (NHMRC clinical trial centre) demonstrated for the first time that fenofibrate therapy in type 2 diabetes significantly and substantially reduced the risk of microvascular-related complications. This project seeks to investigate the effects of fenofibrate, a synthetic ligand for the peroxisome proliferated activated receptor alpha, on impaired ischaemia-mediated angiogenesis and hypoxia tolerance in diabetes mellitus.

3. **The Role of Androgens in Angiogenesis**

   While men are more likely to develop coronary artery disease than women, men are also more likely to have a favourable outcome after a heart attack compared to women. This gender difference after heart attacks, suggests that sex hormones such as the androgens, may play a role in the reparative response after a heart attack.

   In fact, there is evidence from some studies in cells and in animals that androgens increase blood vessel formation. We will study the effects of androgens on angiogenesis and in mobilising endothelial progenitor cells using human cells, animal studies and in a human clinical trial.

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VASCULAR COMPLICATIONS GROUP

THE ROLE OF TRAIL IN REGULATING INSULIN EXPRESSION/SECRETION IN VIVO

Summary

This project will investigate the role of TNF-related apoptosis-inducing ligand (TRAIL) on insulin expression and secretion in normal and diabetic mice.

Background

TRAIL (tumour necrosis factor (TNF)-related apoptosis-inducing ligand) is a protein discovered and named with regards to its ability to promote cell death by binding its specific death receptors. It is now recognized that TRAIL signals can also promote non-apoptotic functions such as cell survival, proliferation, migration and differentiation (reviewed in[1, 2]). We have shown that TRAIL-deficiency in mice promotes atherosclerosis and diet-induced diabetes, suggesting a protective role for this protein in these disease states. Our more recent studies demonstrated a negative association between circulating TRAIL levels and b-cell dysfunction (HOMA-b) in diabetic patients after gastric banding, such that an increase in TRAIL levels after surgery correlated significantly with improved function of b-cells[3]. Reduced HOMA-b was also apparent in our TRAIL-deficient murine models[4]. Furthermore, reduced plasma insulin levels were observed in these mice, associated with reduced insulin expression in pancreatic islets[2]. These suggest that TRAIL may be upstream of insulin expression and secretion. *Whether TRAIL can promote b-cell proliferation, insulin expression and secretion in vivo is unknown.* We will also assess whether TRAIL administration can improve b-cell function in diabetes.

Overview of studies

TRAIL or glucose will be injected into wildtype or *Db/Db* diabetic mice mice at various timepoints followed by assessment of plasma TRAIL levels, insulin and insulin expression in pancreata. We will examine the number of islets, as well as islet size from histology sections, and determine whether TRAIL treatment induces proliferation or apoptosis of b-cells in *vivo.* A range of techniques will be used, including animal handling, biochemistry and immunohistochemistry.

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VASCULAR COMPLICATIONS GROUP

THE ROLE OF ANGIOTENSIN II ON TRAIL TRANSCRIPTION IN VASCULAR SMOOTH MUSCLE CELLS

Summary

This project will investigate the role of Angiotensin II (Ang II) on TRAIL (TNF-related apoptosis-inducing ligand) expression and transcription in vascular smooth muscle cells (VSMCs). We will also identify whether AngII can promote VSMC proliferation or apoptosis via TRAIL.

Background

Activation of vascular smooth muscle cells can promote arterial thickening. We have shown that TRAIL can stimulate proliferation and migration of VSMCs *in vitro* and lead to intimal thickening *in vivo*.

While TRAIL is considered **a** pro-atherogenic molecule early in disease, TRAIL-deficiency in advanced atherosclerosis leads to accelerated atherosclerosis and an unstable plaque phenotype. Comprehension of TRAIL signalling pathways is currently lacking. We have preliminary data that demonstrates that AngII down-regulates TRAIL mRNA expression in VSMCs. We wish to identify the molecular and transcriptional mechanisms for this effect, and also identify whether AngII-dependent TRAIL regulation plays a role in VSMC phenotype. This may provide a mechanism for why TRAIL levels are reduced in patients with cardiovascular diseases.

Overview of studies

Using the full-length and deletion constructs of the human TRAIL luciferase reporter construct, and transient transfection in VSMCs, we will assess the effect of AngII on TRAIL transcriptional activity. We will also identify the transcription factor(s) that may be responsible in this process. Proliferation, migration and apoptosis of VSMCs will be assessed in response to AngII. To identify if AngII-dependent proliferation, migration and apoptosis involves TRAIL, we will use siRNA technology to block TRAIL's effects. We will also look at downstream signalling events including the involvement of TRAIL-receptors. To address the aims, a range of techniques will be used, including cell culture, transient tranfections, luciferase assays, chromatin immunoprecipitation, electrophoretic mobility shift assays, PCR and Western blotting.

References

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VASCULAR COMPLICATIONS GROUP

EFFECT OF TRAIL ON SUPEROXIDE AND ENDOTHELIAL-DERIVED NITRIC OXIDE PRODUCTION

Summary

This project will investigate the role of TNF-related apoptosis-inducing ligand (TRAIL) against oxidative stress-induced endothelial dysfunction, an important clinically relevant process ocurring during atherosclerosis, which manifests as an impairement of the bioactivity of endothelial-derived nitric oxide (EDNO).

Background

TRAIL is a protein discovered and named with regards to its ability to promote cell death by binding its specific death receptors. However, TRAIL's functions are pleiotrophic since we recently discovered a novel protective function for TRAIL in cardiovascular disease (CVD)1-3 and diabetes2. Notably, circulating TRAIL levels are significantly reduced in patients with CVD4 and reduced TRAIL levels independently predict cardiovascular events and mortality5. Despite evidence for a protective role of TRAIL in CVD, the molecular mechanisms by which it exerts its beneficial actions are currently unknown. Our novel preliminary data support the hypothesis that TRAIL targets the vascular endothelium, reducing vascular oxidative stress and enhancing EDNO bioactivity, thereby affording protection against endothelial dysfunction and atherosclerosis.

Overview of studies

Using unique murine models and *in vitro* cellular assays, we will identify the effect of TRAIL on superoxide and EDNO production. In collaboration with the Inflammation Group at the HRI, a number of biochemical techniques will be used including superoxide measurement in tissues/cells by HPLC; EDNO bioactivity measured using enzyme immunoassay kits. Nitric oxide formation in aortic segments will be quantified using electron paramagnetic resonance (EPR) spin-trapping.

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