

# William Harvey Research Foundation

## Report of Research Grant Activities 2004 – 2006



*“Supporting vascular research for the discovery of new treatments”*

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## Cardiovascular Medicine Projects

**1. Project:**        **Therapeutic approaches for acute myocardial infarction**

**Grant Holder:**   **Professor Chris Thiemermann**  
**Researcher:**     **Dr. Michelle McDonald**

Since the turn of the last century, cardiovascular disease has been the leading cause of death in the United Kingdom in every year but one (1918). The goal of this project was to find new therapies to reduce the damage caused by heart attacks (using an experimental model of acute myocardial infarction). During this work it was discovered that specific endogenous substances (PPAR-gamma ligands and hydrogen sulphide) protect the heart against the tissue damage caused by reduced blood flow (termed "ischaemia") and its subsequent restoration (termed "reperfusion"). It was found that the recently discovered gaseous mediator hydrogen sulphide is produced during ischaemia and reperfusion of the heart in sufficient quantities to protect the heart against injury such that preventing its formation increases the degree of cardiac injury. Based on these findings, future research will investigate the utility of exogenous hydrogen sulphide (or molecules which activate these protective mechanisms) as agents for protecting the heart against the injury associated with coronary artery bypass graft surgery and/or heart transplantation.

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**2. Project:**        **Glycogen synthase kinase in inflammation and shock**

**Grant Holder:**   **Professor Chris Thiemermann**  
**Researchers:**   **Dr. Marika Collin**  
                          **Dr. Laura Dugo**

The medical syndrome of shock can be defined as a "*progressive failure of the circulation to provide blood and oxygen to vital organs of our body*". The most common cause of shock is the contamination of blood with bacteria resulting in systemic infection and ultimately shock (septic shock). Another important cause of shock is the severe blood loss associated with trauma (haemorrhagic shock). Despite improvements in intensive care medicine, the mortality of shock remains very high, and there is still a great need for new approaches to improve therapy and outcome of patients

with shock. These investigations showed that commercially available inhibitors of the enzyme glycogen synthase kinase-3 (GSK-3) reduce the degree of systemic inflammation and organ injury associated with septic and haemorrhagic shock. It was also discovered that insulin inhibits the activity of GSK-3, which may explain the beneficial effect of insulin in septic shock as it also reduces the morbidity and mortality of these patients. Based on these findings it was concluded that inhibitors of the activity of GSK-3 have potential as novel treatments for inflammation and shock. So studies were also initiated to determine whether inhibitors of GSK-3 exert beneficial effects in models of local inflammation.

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**3. Project:**        **HDL and other lipids in shock**

**Grant Holder:**   **Professor Chris Thiemermann**  
**Researcher:**     **Maha Abdelrahman (PhD-student)**

Due to the relatively high mortality of patients suffering from septic shock, there is an urgent need for new approaches to improve therapy and outcome. Hence, identification of completely novel ways of managing these patients is an important element of research in this field. A number of studies have shown high density lipoprotein complexes (HDL) have anti-inflammatory properties. So its potential to reduce the systemic inflammation and organ injury associated with shock of various aetiologies was investigated. This revealed HDL as a very promising new approach. In addition, HDL was found to reduce the tissue injury caused by reduction of blood flow (ischaemia) and excessive local inflammation (e.g. colitis). Because HDL comprises a number of different lipids further studies were initiated to evaluate the actions of a variety of lipids and HDL-moieties in order to identify the fraction within the HDL (for example lysophosphatidylcholine), which accounts or at least importantly contributes to the observed beneficial effects of HDL. The overall goal of this project is to identify a fraction within HDL that ultimately can be developed as a therapeutic remedy for shock and inflammation.

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#### **4. Project: Vascular reactivity alterations in cardiovascular disease**

**Grant Holder: Prof. Amrita Ahluwalia**

A profound fall in blood pressure and altered vascular function is a general feature of septic shock. The goal of this work was to investigate the mechanisms of vascular dysfunction in sepsis. A recognised consequence of bacterial infection is the induction of a state of vascular hyporesponsiveness to nitric oxide (NO) released by agents that generate NO in the vessel wall. This is due to NO-induced desensitisation of guanylate cyclase as a result of excessive production of NO by the inducible isoform of nitric oxide synthase (iNOS). Work on this project showed that this desensitisation did not occur in large arteries that lacked the endothelial isoform of nitric oxide synthase (eNOS) as a result of gene deletion. Detailed investigations showed this was because iNOS was not induced when eNOS was absent. Subsequent studies have indicated an interplay between NO and prostacyclin derived from cyclo-oxygenase-2 (COX2) in the regulation of soluble guanylate cyclase in the blood vessel wall. Future studies will investigate the interaction of these mediators in the regulation of blood vessel sensitivity in sepsis with the aim of providing insights that will help the restoration of normal vascular function.

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#### **5. Project: Research on red wine polyphenols**

**Grant Holder: Professor Roger Corder**  
**Researcher: Noorafza Khan**

Population studies over the past twenty years have shown that daily consumption of 2 – 3 glasses of red wine reduces coronary heart disease by approximately 50%. The aim of this project was to investigate whether a specific component of red wine could explain its vascular protective properties. Initial investigations showed that red wine extracts inhibit endothelial cell synthesis of endothelin-1, a key mediator in cardiovascular disease. Subsequent studies focused on purifying the active ingredient from red wine extracts responsible for inhibiting endothelin-1 synthesis. This identified procyanidins (a type of flavonoid polyphenol) as the main vasoactive component of red wine. To see if wines of different origins all have similar levels of procyanidins, red wines from different countries and regions were analysed. Studies of Sardinia and southwest France showed red wines with particularly

high concentrations of procyanidins are associated with areas of reduced heart disease and increased longevity. More recent research has used microarray analysis to characterise all the genes in endothelial cells regulated by procyanidins. This has identified a highly integrated protective response, which merits further research in a number of areas. Research on this project has highlighted the need to test whether treatment of individuals with procyanidin-rich products, such as grape seed extract, can reduce symptoms of heart disease.

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#### **6. Project: Mechanisms of coronary calcification**

**Grant Holder: Professor Roger Corder**  
**Researchers: Liz Wood**

Arterial calcification is a prominent feature of atherosclerosis and is associated with an increased risk of cardiovascular events. Osteoprotegerin is a cytokine that has recently been implicated in the regulation of vascular calcification. This project evaluated the relationship between plasma osteoprotegerin (OPG), inflammatory biomarkers (high-sensitivity C-reactive protein, hs-CRP; interleukin-6, IL-6), coronary artery calcification (CAC), and cardiovascular events in patients with type 2 diabetes. A total of five hundred and ten patients with type 2 diabetes free of symptoms of cardiovascular disease were evaluated by CAC imaging. Patients were followed up for cardiovascular events (cardiac death, myocardial infarction, acute coronary syndrome, late revascularization, and nonhaemorrhagic stroke). Significant CAC was seen in 43.6% of patients. A key finding of these investigations was that OPG levels were significantly elevated in patients with increased CAC. In multivariable analyses, OPG retained a strong association with elevated CAC scores after adjustment for age, gender, and other risk factors. In comparison levels of hs-CRP and IL-6, two commonly used markers of vascular inflammation, were not related to the degree of CAC or short-term events. An important conclusion from this study was that a high proportion of asymptomatic diabetic patients have significant subclinical atherosclerosis. In addition, of the biomarkers studied, only OPG predicted subclinical disease. Hence, measurement of plasma OPG may help in the identification of patients with type 2 diabetes that have a high-risk of developing coronary artery disease.

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**7. Project: Techniques to measure large and peripheral artery function.**

**Grant Holder: Dr. Martin Carrier**

The vasodilator molecule nitric oxide (NO) is continuously synthesised by the vascular endothelium to regulate vascular tone and blood pressure. It plays a central role in vascular physiology and pathology, but studies of its functional activity in experimental and clinical situations are severely limited by difficulties in measuring it routinely. Over several years research in the WHRI has been undertaken to develop a simple, non-invasive technique for assessing NO bioactivity and function. The hypothesis underlying the technique used in this research is that NO modifies the reflection of pressure and flow waves within the arterial system, and that this can be assessed by examining the blood pressure pulse waveform as it is sensitive to wave reflections, with and without modification of NO bioactivity. This research has shown that an alteration in NO synthesis changes the shape of the peripheral pulse wave. Increased synthesis decreases the height of the dicrotic notch, relative to the overall height of the wave ("relative height of the dicrotic notch", RHDN), whilst decreased synthesis has the opposite effect. These influences may also depend on alteration of vascular wave reflections. Hence, this project tested whether changes in RHDN could provide a simple specific non-invasive index of NO bioactivity. Data obtained during this project confirmed the role of NO in determining changes in RHDN.

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**8. Project: Cyan analyser flow cytometer equipment grant**

**Grant Holder: Dr. Anthony Mathur**

Identification of cells using specific labelled antibodies with fluorescence detection, and recognising changes in function by alterations in cell surface expression of proteins, is a key technology for studying disease mechanisms research. Purchase of a Dako Cyan flow cytometer has enhanced the scope for doing this type of research, for instance in studying mechanisms of inflammation. This equipment will also have considerable utility for advancing the stem cell research currently being undertaken, and also provides new opportunities for other research groups in the William Harvey Research Institute.

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## Inflammation Projects

### 9. Project: The Role of Glutamate in Blood-Brain Barrier Breakdown in Models of Multiple Sclerosis

**Grant Holder: Prof. Rod Flower & Dr. Chris Bolton**

Breakdown of the blood-brain barrier (BBB), as part of disease onset and development, is a common feature of the human demyelinating disease multiple sclerosis (MS) and the experimental counterpart allergic encephalomyelitis (EAE). Loss of normal BBB function allows destructive cells and inflammatory mediators access to the central nervous system resulting in damage to nerve fibres and, ultimately, impaired motor function that has profound effects on movement and co-ordination. One principle candidate implicated in BBB breakdown is the amino acid glutamate that triggers a variety of reactions culminating in irreversible damage to motor nerve fibres. The current work has focused on the ability of glutamate to mediate BBB damage via specific receptor activation coupled to the production of the neurotoxic free radical peroxynitrite. In particular, the studies showed the addition of glutamate to cells derived from the BBB cause abnormal permeability changes that can be dramatically reversed by drugs that limit receptor activation and peroxynitrite production. The importance of glutamate and peroxynitrite in mediating BBB damage has also been demonstrated in EAE. Administration of the catalytic drug FeTPPS that inhibits glutamate-generated peroxynitrite production markedly suppressed the neurological episodes associated with EAE. The work has highlighted the involvement of glutamate in BBB breakdown and the development of EAE, and hence suggests a role for the molecule in the pathogenesis of MS. These findings will enable the testing of new strategies to manage MS.

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### 10. Project: Glucocorticoid, Annexin 1 and the control of adaptive immunity

**Grant Holders: Prof. Mauro Perretti**

This project has focused on the annexin 1 system and on its ability to reduce the inflammatory response to external insults (e.g. infection) and internal anomalies (e.g. ischaemia, which occur in heart attack or stroke). Together with other pathways, the annexin 1 system is crucial to the well being of our body, and alterations lead

to disease (over-shooting of the inflammatory reaction). In line with this hypothesis, absence of annexin 1 exacerbates these responses. Over the last two years investigations have characterised the way the natural protein, annexin 1, acts at its receptor, termed FPRL-1, such to form a key/lock pair. The project has addressed several aspects of the annexin 1 system, including a) study the effect of clinically effective drugs [the glucocorticoids] on the expression of annexin 1 and its receptor; b) analysis of annexin 1 mimetics with the aim of discover leads for new therapeutic development; c) exploitation of the clinical impact of this research. Studies on the last of these areas has revealed the altered expression of the annexin 1 system in white blood cells of patients suffering from of rheumatoid arthritis and cystic fibrosis; current work focuses on vasculitis and lupus erythematosus. Further research in this area is hoped to lead to medicines that mimic the effects of annexin 1 as these may be better than existing ones for two reasons. Firstly, they are likely to have a reduced profile of side effects, because they will be mimicking the way our body controls inflammation in health. Secondly, annexin 1 mimetics will reproduce some of the anti-inflammatory effects of glucocorticoids without the problems associated with these medicines during long-term use.

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### 11. Project: Regulatory processes in gastrointestinal inflammation

**Grant Holder: Professor Brendan Whittle**

A number of serious disorders that can affect the gut may have common links to inflammatory and vascular diseases, or may result from medicines designed to treat those illnesses. One class, inflammatory bowel diseases (IBD), afflicts 1 in every 400 people within the United Kingdom. This research is seeking biotargets for new drugs that can directly regulate the inflammatory disease process in the gut. The focus of these efforts is on the interplay between the local vascular and inflammatory substances and cells in the small blood vessels that could cause IBD. Attempts are also being made to exploit the body's own defensive mechanisms to develop new drugs to prevent and heal IBD and eliminate drug side effects. One pivotal component of inflammatory processes is the nuclear factor, *NFκB*, which promotes the expression of relevant pro-inflammatory genes. Working in collaboration with Professor Thiernemann, who has recently identified a novel approach to the regulation of *NFκB*, we identified the therapeutic potential for the treatment of IBD of

modulating *NFκB* using specific inhibitors of the enzyme pathway known as *glycogen synthase kinase-3β*. Other cell-based targets such as the newly discovered *histamine H<sub>4</sub> receptor* that is located specifically on cells of the immune system are also been explored. This has shown that blockade of these histamine receptors can actively reduce extent of the inflammatory response such as that in the gut. All of these findings are increasing understanding of the vascular and injurious factors that can be targeted by novel therapeutic agents to reduce the gut inflammation that occurs either naturally, or as a side effect of some anti-inflammatory drugs.

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## Joint and Bone Research Projects

**12. Project:**     **Inflammation Research Fellowship  
Auto-immune diseases research  
Pharmacological manipulation of  
inflammation in vivo.**

**Grant Holder:**   **Dr. Michael Seed**  
**Researchers:**   **Mrs. MR Jones & Ms S. Gor**

These projects have built on the success of the previous years. Work has concentrated on the involvement of a molecular pathway that is known to be intimately involved in inflammation, *NFκB*. One important enzyme in this complex, *IKKα*, has been investigated in detail using an experimental model that has been genetically engineered to inactivate this enzyme (kindly donated by Prof. M Karim, University of San Diego). Previous work on this model had shown that *IKKα* was important in pathways involved in the resolution of acute inflammation. Work on this project has shown the opposite to be true in inflammation resulting from hypersensitivity similar to autoimmune disease, in that hypersensitivity responses were reduced. Investigations of immune cells in culture show they still react to non-self, but when exposed to hypersensitivity antigens their responses are much reduced, as is one of the prime signals in these events, dendritic cell interleukin-12. Unexpectedly it appears to be very important in the T-lymphocyte response to antigen. This means that this molecule plays a pivotal role in the switch between the resolution of acute inflammation to a chronic non-resolving inflammation in autoimmune disease. Thus inhibitors of this enzyme may prove of benefit in autoimmune disease. Further inflammation research has included characterisation of the actions of diosamine.

**13. Project:**     **Osteoclast behaviour research  
Calcium metabolism in bone  
disease**

**Grant Holder:**   **Professor Iain Macintyre FRS**  
**Researchers:**   **Dr. Lucia Mancini**

Two main areas of bone metabolism research have been undertaken over the past few years. Firstly, studies of the anti-arthritic action of calcitonin in experimental models; this has shown that calcitonin has a marked suppressive effect especially in combination with prednisolone such that experimental arthritis can be completely inhibited. The second area has been the actions of parathyroid hormone on bone cells. Parathyroid hormone is the only agent that can reverse the bone loss found in osteoporosis and it is important to investigate whether this action depends on nitric oxide. Experimental studies of osteoblasts have investigated the regulation of nitric oxide synthesis. The hypothesis being explored is that increased NO synthesis is either involved in the actions of parathyroid hormone or promotes its actions. These studies may lead to new approaches to the management and treatment of osteoporosis.

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## Training Programmes

**23. Project:** Masters of Research:  
Mechanisms of Vascular Disease

**Grant Holder:** Professor Roger Corder

**Students:** Syed Amir Mahdi Abedi  
Paul Armstrong, Alicia Murcia,  
Nicola Pearson.

**MRC MRes/Phd Students with Supplementary Support from WHRF:**

Stephanie Francis, Mark Potheary,  
Paul Caton, Oliver Murch

The William Harvey Research Institute established a Masters of Research training programme in 2002 to teach an integrated approach to research that combines traditional pharmacological methods with modern techniques of molecular biology, genomic and proteomic research. This is combined with greater training on the causes and mechanisms of diseases. The MRes course provides a foundation to 3-year PhD training. The students passing through this programme have shown greater productivity and increased ability to develop their PhD projects as individuals: key criteria for becoming successful scientists. The William Harvey Research Foundation has played a key role in enhancing research training by giving this a high priority for funding.

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