Blood clot structure plays a role in predisposition to atherothrombotic disease, as compact clots are associated with premature and more severe cardiovascular disease. Some genetic variants of clotting factors have been associated with an increased risk of thrombosis by mechanisms that may involve alteration in clot structure. In addition to genes, environmental factors, such as obesity, diabetes and smoking can also predispose to cardiovascular disease. Diabetes is associated with alterations in clot structure and increased thrombosis potential, which may be related to increased glycation of clotting factors, secondary to high plasma glucose.

The bulk of the current work is focussed on analysing the effects of genetic variations in clotting factors (mainly fibrinogen and factor XIII) on clot structure and function using recombinant techniques. The effect of diabetes on clot structure is under investigation in a longitudinal study in patients with poorly controlled type 1 diabetes before and after improving glycaemic control. In addition to diabetes, other endocrine conditions, known to be associated with increased atherothrombotic risk, are studied, including thyroid disease and polycystic ovary syndrome.

Modulation of clot structure and fibrinolysis by therapeutic agents, including oral hypoglycaemic and antplatelet drugs are also under investigation, in collaboration with the University of Hull and the University of Sheffield. This may help to develop new treatment strategies aiming to reduce the risk of atherothrombotic events, particularly in high risk subjects. Techniques used include cell culture, site-directed mutagenesis, mammalian and bacterial expression systems, permeation analysis, turbidity measurement, confocal microscopy, electron microscopy, magnetic tweezers and mass spectrometry.

Funding
Department of Health
British Thyroid Foundation
National and International Collaborators
Professor John Weisel (University of Pennsylvania, USA)
Professor Steve Atkin (University of Hull, UK)
Professor Simon Pearce (University of Newcastle, UK)
Dr. Robert Storey (University of Sheffield, UK)
Dr. Mark Strachan (University of Edinburgh, UK)
Dr. Azfar Zaman (University of Newcastle, UK)

More information:
http://www.cardiovascular.leeds.ac.uk/staff/Ajjan_R/

Representative publications
FXIII and fibrin clot structure/function

Fibrin is the main protein constituent of the blood clot. Conversion of fibrinogen to fibrin by thrombin involves polymerisation of the fibrin units into protofibrils which associate laterally to form fibres. The fibres branch out and form a three-dimensional network which acts as a scaffold for the thrombus and with which cells interact. This structure is stabilised by factor (F)XIII, which is a protransglutaminase that on activation by thrombin introduces covalent cross-links in fibrin.

My research is focussed on how cross-linking by activated FXIII influences the structure of the clot and its resistance to breakdown. The fibrin clot is not a passive substrate for FXIIIa; it is actively involved in the regulation of FXIII activity. We express mutants of recombinant fibrinogen and FXIII to characterise areas of molecular interaction that regulate cross-linking activity. There is evidence to suggest that cross-linking reaction itself changes the structure of the clot, but to what extent and by which mechanisms is unknown. Our aim is to investigate mechanisms underpinning this and those related to the resistance of the clot to fibrinolysis.

Post-translational modification
Also post-translational modifications have major potential effect on the structure and function of the fibrin clot. Such modifications are often disease specific, modifiable by lifestyle changes or drugs, and hence of major interest therapeutically. Protein glycation is a risk factor for thrombosis in patients with diabetes. We investigate whether glycation of clot proteins alter their function to increase risk. We also investigate the effects of homocysteine on clot proteins. Elevated homocysteine associates with dietary changes or genetic variation in the vitamin B dependent metabolism pathways and associates with an increased risk for thrombosis. We hypothesise that effects on clot formation may have a role. A third area of research involves the characterisation of proteolytic modifications of plasmin inhibitor. Cleavage of this inhibitor leads to several variants, the functions of which are not yet fully understood.

Funding:
British Heart Foundation, Department of Health, Medical Research Council, Wellcome Trust, Diabetes UK
Collaborators:
John Weisel (Philadelphia)
Susan Lord (Chapel Hill)
Charles Greenberg (Duke)
Martin Guthold (Wake Forrest)

More information:
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[4] Representative publications
Genetic and Environmental Risk Factors for Cardiovascular Disease

Research is focussed on identification and functional analysis of genetic and environmental risk factors for cardiovascular disease (CVD) and clustering of cardiovascular risk factors in subjects with type 2 diabetes mellitus. Particular emphasis of research is on haemostatic and inflammatory cardiovascular risk factors and their influence on the thrombotic component of cardiovascular disease.

Analysis of plasma proteins involved in determination of clot structure and function: We have shown that genetic factors account for ~0.30% of variance in clot structure and clot lysis phenotypes and that these phenotypes are related to cardiovascular risk assessed by presence of the metabolic syndrome. We have carried out a proteomic analysis of plasma clots and identified a number of novel clot proteins (unpublished data) which are the subject of ongoing functional analyses. Identification of the genetic and environmental factors influencing clot structure/function may further our understanding of the factors predisposing to CVD.

The role of complement and complement activation in relation to thrombosis: There is increasing evidence to indicate that activation of complement plays a functional role in the pathogenesis of CVD. We have shown that C3 is independently associated with CVD after accounting for classical cardiovascular risk factors, whilst C-reactive protein, a more commonly analysed inflammatory biomarker, is not. Furthermore we have identified complement C3 as a novel fibrin clot component, suggesting a direct influence of C3 on thrombus formation. Projects are currently underway to characterise the influence of C3 on fibrin structure/function and thrombosis.

Collaborators:
Paul Bray (Huston), Tim Spector (London), Khalid Naseem (Bradford), Jerry Thomas (York)

Funding: British Heart Foundation, Heart Research UK

More information: http://www.leeds.ac.uk/medhealth/light/staff/carter_a.html

Representative publications
Atherosclerosis and Diabetes
MULTIDISCIPLINARY CARDIOVASCULAR RESEARCH CENTRE (MCRC)

Colin Fishwick
BSc
PhD (Liverpool)
Reader in Organic Chemistry (2006 -)
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Design and Synthesis of New Small-Molecule Inhibitors

Our research is focused on the application of computer-aided molecular design methods to the production of potent and selective small-molecule inhibitors of a range of therapeutically important enzymes and receptors (Figure 1).

Although we use a wide variety of in silico methods in our work, of particular importance are virtual high-throughput screening (vHTS) and the de novo molecular design program SPROUT (developed by our collaborator Prof. Peter Johnson). In collaboration with several groups, both nationally and internationally, we are presently using computational and synthetic techniques to produce small-molecule inhibitors for use in the treatment of both infective- and non-infective diseases, respectively. Within the infective diseases arena, systems presently under study include a range of enzymes derived from pathogens including those involved in bacterial cell-wall biosynthesis, and in the biosynthesis of nucleic acids from malarial parasites. Research on the production of inhibitors of enzymes associated with non-infective diseases includes work on the development of new anti-thrombotics, development of new leads for the treatment of Type II diabetes, and new anti-cancer compounds, respectively. Representative examples of current projects are listed below.

Probing the potential of blood-clotting FXIIIA in the treatment of thrombosis
Myocardial infarction (MI) occurs when a platelet-rich fibrin plug forms on the inner surface of a coronary artery. This obstructs cardiac blood flow causing ischaemia and tissue damage. The sites of thrombus formation are often ruptures in the caps of arterial plaques which have developed over many years. Factor XIIIA is an enzyme which renders blood clots resistant to fibrinolysis by cross-linking fibrin chains to adjacent chains and to fibrinolytic inhibitors.

In vivo perfusion studies have shown that fibrinolysis of clots is promoted by inhibitors which covalently inactivate FXIIIA. We are using the SPROUT software to identify non-covalent inhibitors of FXIIIA directed towards (i) the active site cleft or (ii) residues surrounding this cleft that must be displaced for FXIIIA to be activated. This work is in collaboration with Prof. Peter Grant and Dr. Richard Pease, and is funded by a grant from Heart Research UK.

Dihydroorotate dehydrogenase inhibitors as new potential antimalarials.
Malaria continues to represent a major threat to world health infecting between 300-500 million people annually and causing up to two million deaths. The disease results from infection by parasites belonging to the Plasmodium species and is transmitted by the female mosquitoes of the Anopheles genus. Of the four species of parasite that infect humans, Plasmodium falciparum is responsible for the majority of fatalities. Although prophylactic treatments are available, resistance to commonly employed anti-malarial drugs (for example chloroquine, pyrimethamine, and atovaquone) is widespread. There is, therefore, an urgent need for the development of new drugs that can control infection which can also act on previously unexploited biological targets. Dihydroorotate dehydrogenase (DHODH) is an essential enzyme, involved in the biosynthesis of pyrimidine nucleobases within the parasite. Using the SPROUT de novo molecular design method in combination with chemical library synthesis, we have developed several new DHODH inhibitors, some of which show promising antiparasitic activity (Figure 3). This work has been funded via a studentship (to Prof. Peter Johnson) from the Wellcome Trust, and in collaboration with Drs. Glenn McGonkey and Mark Parsons.

Dihydroorotate dehydrogenase as Antiplasmodial Compounds,
Fishwick, Lars Hesse, James R. Horton, and Peter Johnson. Macrocyclic Inhibitors of the Bacterial Cell Wall Enzyme MurD.

Figure 1 discovery of lead molecules

Figure 2 Structure of FXIIIA dimer

Figure 3 SPROUT designed DHODH inhibitor
Towards an effective anti-Alzheimer’s treatment: application of vHTS and de novo design to the development of new BACE inhibitors
Alzheimer’s Disease (AD) accounts for the majority of dementia diagnosed in patients after the age of 60. The formation of insoluble extracellular amyloid plaques by the accumulation of amyloid β-peptide (Aβ) is one of the key pathological features in AD brain. Aβ is generated by the proteolytic cleavage of the β-amyloid precursor protein (APP). β-Secretase (β-site APP cleaving enzyme, or BACE-1) is the first of the two enzymes responsible for the sequential processing of APP and is an attractive target for the development of drugs to combat the disease (Figure 4). We are applying a range of in silico drug design methods to X-ray crystal structures of BACE in order to identify new small molecule BACE inhibitors. Several of these are effective at lowering cellular levels of Aβ and may be represent promising leads for further development. This work is in collaboration with Prof. Nigel Hooper and is funded by a major project grant from the Alzheimer’s Research Trust.

Funding: BBSRC, MRC, EEC, Alzheimer’s Research Trust, Heart Research UK
More information:
http://www.cardiovascular.leeds.ac.uk/staff/Fishwick

Representative publications
Type 2 diabetes is characterised by the presence of underlying insulin resistance and associated clustering of inflammatory atherothrombotic cardiovascular risk. The development of insulin resistance is strongly related to the presence of intra-abdominal obesity which is associated with decreased insulin signalling in the adipocyte and other cells. The fat-filled adipocyte changes associated with obesity and insulin resistance are accompanied by thrombotic risk clustering evidenced by suppression of fibrinolysis due to elevated PAI-1 and increased levels of coagulation Factors VII, XII and fibrinogen. As the disease process cycles from euglycaemic insulin resistance towards type 2 diabetes, the development of hyperglycaemia has additional effects on vascular risk including endothelial cell function and through post translational modifications of fibrin.

Work from our unit has identified both genetic and environmental contributions to fibrin structure function (Circulation 1997; 96: 1424-31; Blood 1999; 3: 906-8). We were the first to identify and characterise the effects of FXIII/Val34Leu on fibrin structure/function (Blood 2000; 96: 988-95) and cardiovascular risk (Blood 2002; 100: 743-54) and since then we have reported early proof of principle of small molecules and RNA aptamers that interfere with fibrin formation. Results from these studies have been published in Blood, Lancet, Circulation, JBC and ATVB.

More recently we have developed mechanistic studies evaluating functional characteristics of FXIII/fibrin interactions which drive thrombosis, (Blood; 2007; 110: 902-7, Blood 2008; 111: 643-650) and we have early proof of principle of small molecules and RNA aptamers that interfere with fibrin formation. Results from these studies have been published in Blood, Lancet, Circulation, JBC and ATVB.

Figure 1 Fibrin generated from fibrinogen from a non-diabetic (upper panel) and diabetic (lower panel) subject. Poorly controlled diabetes is associated with the formation of a dense tightly packed fibrin clot with thin fibres that is resistant to proteolysis by the fibrinolytic system.

Fig 2 Effects of improving glycaemic control on fibrin rigidity before (red) and after (black) intensive glucose management.

Funding: British Heart Foundation (Programme Grant Holder), Department of Health, Diabetes UK.

Collaborations: Spector (St Thomas’, London), Weisel (Penn, PA, USA), Marx (Ulm, FDR), Medcalf (Monash, Melbourne, Aus), Bouger (Munich, FDR), Sheffield Biomedical Research Unit (Crossman, Heller, Storey), Jackson, Bristol UK, Fishwick, Leeds

More information:
http://www.cardiovascular.leeds.ac.uk/staff/Grant_PJ/

Representative publications:
Genetic & Environmental Risk factors for Cardiovascular Disease in South Asians

South Asians (subjects who themselves or their grandparents have originated from India, Pakistan or Bangladesh) are more prone to ischaemic heart disease and ischaemic stroke. These conditions run a more malignant course in South Asians and hence result in increased mortality. The cause for this increased morbidity and mortality is not understood because increased prevalence of the traditional risk factors i.e. type 2 diabetes, hypertension, dyslipidaemia, visceral obesity, insulin resistance and few novel risk factors do not fully explain it.

My aim has been to investigate differences in genetic and environmental risk factors, thrombotic factors, fibrin structure and function between South Asians (with or without cardiovascular disease) and Whites and their relationship with insulin resistance.

We have demonstrated that plasma levels of clotting factors, fibrinogen and plasminogen activator inhibitor -1, markers of chronic subclinical inflammation, ‘C’ reactive protein and complement C3, are higher in South Asians. Additionally plasma levels of tissue plasminogen activator, fibrinogen, plasminogen activator inhibitor -1, factor VII antigen, von Willebrand factor, factor XIII B subunit with insulin resistance and plasminogen activator inhibitor -1 was also observed in South Asians with coronary artery disease.

South Asians with Computerised Tomography confirmed ischaemic stroke have raised levels of fibrinogen, tissue plasminogen activator and von Willebrand factor as compared to apparently healthy South Asians. There are gender differences in thrombotic factors and factor VII antigen levels are higher and plasminogen activator inhibitor -1 lower in South Asian women with stroke (Figure 1). Moreover, first degree relatives of South Asians with ischaemic stroke have increased insulin resistance and raised levels of tissue plasminogen activator as compared to controls.

Metabolic syndrome is increased in South Asians and complement C3, tissue plasminogen activator as well as HOMA insulin resistance independently predict it in this high risk group. Moreover, raised liver enzyme alanine aminotransferase which is a marker of fatty liver in the absence of alcoholism is a feature of metabolic syndrome in South Asians (Figure 2). We have also examined the common polymorphisms of fibrinogen, Factor VII, Factor XIII and plasminogen activator inhibitor -1 in relation to ischaemic stroke in South Asians.

We continue to investigate novel atherothrombotic risk factors and their common polymorphisms in South Asians.

Funding:
Stroke Association

More information:
http://www.cardiovascular.leeds.ac.uk/staff/kain_k/

Representative publications:


Regulation of haemostasis: balance of coagulation and fibrinolysis

My research examines the regulation of coagulation and fibrinolysis and how these systems intertwine in thrombus formation and dissolution. Thrombosis is a major cause of mortality in the Western world, accounting for 40% of all deaths. Haemostasis, the arrest of blood flow from a vessel, is exerted by the tight regulation of coagulation and fibrinolysis under normal circumstances a balance between these two systems exists. Upon injury, a fibrin-platelet plug forms reducing blood loss. The fibrinolytic cascade then dissolves the fibrin-platelet plug allowing blood to flow freely through the vessel. An imbalance in these systems may lead to either haemorrhage or thrombosis.

Components of the coagulation cascade circulate in blood as inactive zymogens that are activated sequentially by the preceding enzyme in the cascade and culminate in generation of the enzyme thrombin. This serine protease activates platelets and converts fibrinogen to fibrin allowing thrombus formation. Very little thrombin is required to form a clot but large quantities are generated through amplification of the coagulation cascade and my work has shown that a substantial amount remains associated within the thrombus itself. Thrombin is a complex enzyme which functions in many processes such as fibrinolysis, crosslinking, signaling and anticoagulation. These actions are regulated by several cofactors which alter the specificity of thrombin. A main focus of my research is to address why so much additional thrombin is formed during blood coagulation, why it persists in thrombi and its main physiological targets.

Platelet dense granules have recently been discovered to contain an inorganic molecule, polyphosphate (Figure 1), which is released upon platelet activation by thrombin.

Our manuscript in PNAS describes the role of platelet polyphosphate in acceleration of coagulation and downregulation of fibrinolysis (Figure 2); the effects centre around thrombin formation and my recent studies have shown that polyphosphate interacts directly with exosite II on thrombin. the physiological importance of polyphosphate in haemostasis and its specific interactions are a current focus of my laboratory.

More information: http://www.cardiovascular.leeds.ac.uk/staff/Mutch_N_/representative_publications


Mechanisms of Thrombosis

Introduction
The haemostatic system plays a central role in the pathogenesis of acute coronary syndromes (ACS). Release of procoagulant debris following plaque rupture precipitates a series of events which activate both the coagulation cascade and circulating platelets, to create a platelet rich thrombus, vascular occlusion and the acute complications of athero-thrombosis such as myocardial infarction (MI) and ischaemic stroke. Cross-linked fibrin provides the principal structural support to the thrombus. The enzymatic reactions critical for determining its structure, stability and susceptibility to fibrinolysis are: i) conversion of fibrinogen to fibrin by thrombin (inducing spontaneous polymerisation of fibrin monomers into the fibre network) ii) the formation of covalent cross-links within this fibrin network and (iii) the cross-linking of plasmin inhibitor (α2-antiplasmin) to fibrin by FXIIIa. Factor XIII is a tetramer composed of two A-(possessing catalytic site) and two B-(carrier proteins) subunits, which is activated by thrombin-induced cleavage of the Arg37-Gly38 peptide bond (enhanced by co-factor activity of polymerised fibrin), and calcium dependent dissociation of the A-and B-subunits.

Research Interests
My research is focused on understanding mechanisms of thrombosis by characterising the nature of end-stage coagulation protein-protein interactions, regulatory mechanisms and the influence of post-translational modifications of proteins (by glycation or homocysteinlyation) in determining a blood clots resistance to fibrinolysis. In addition my research includes the development of novel inhibitors to thrombosis as potential therapeutics.

Research Techniques
Techniques employed include site-directed mutagenesis and recombinant expression of proteins involved at the end-stage coagulation system, surface plasmon resonance and functional characterisation of proteins in relation to clot formation and fibrinolysis. Functional characterisation of clot formation is performed with a variety of methods including fibrinolysis by Chandler’s loop, fibrinolysis by confocal microscopy and measurements of factor XIII activity using intravital microscopy.

Collaborators:
Prof Muriel Maurer, University of Louisville, USA, Dr Colin Longstaff, NIBSC, UK

Funding:
British Heart Foundation
Diabetes UK

More information:
http://www.leeds.ac.uk/medhealth/light/staff/philippou_h.html

Representative publications
Understanding the Pathophysiology of Vascular Disease Associated With Insulin Resistance

I am interested in understanding the mechanisms of vascular disease associated with insulin resistance. My research in this area takes three approaches:

1) The role of circadian/diurnal rhythm disruption in the development of insulin resistance and cardiovascular disease, with particular focus on the molecular clock.

The 24-hour environment of light and dark governs life on earth. Adaptation to this 24-hour cycle enables an organism to anticipate environmental fluctuations over the day and to optimise the timing of relevant biological processes. As such, most physiological processes are coordinated to this 24-hour cycle with a diurnal rhythm. Recent evidence has uncovered the molecular clock as a key regulator of this diurnal variation. One of the components ‘Clock’ has been identified as instrumental in the development of the cardiovascular risk factors associated with the metabolic syndrome in murine models. We have shown a consistent association between common polymorphisms in the Clock gene and the metabolic syndrome in man, in two population studies and have ongoing clinical studies to develop this work further. We are exploring the role of ‘Clock’ in the development of endothelial dysfunction, utilizing a tissue specific model.

2) The role of the sympathetic nervous system in the development of vascular disease associated with insulin resistance, utilizing the technique of microneurography.

The only direct method for assessing the sympathetic nervous system in humans is microneurography, as this allows ‘real-time’ assessment of muscle sympathetic nerve activity (MSNA) to be made under both resting and dynamic conditions. MSNA measures the post-ganglionic sympathetic outflow destined to supply the peripheral vasculature and which is therefore important in the regulation of systemic haemodynamic homeostasis. In recent studies from the microneurography unit we've demonstrated that insulin resistant subjects without type 2 diabetes have elevated levels of resting MSNA and that uncomplicated T2DM have extremely elevated levels of resting MSNA.

3) Investigating the role of prothrombotic, haemostatic factors in vascular disease and how these are modified by insulin resistance, focusing on fibrin structure/function.

The formation of a fibrin clot is a major step in the atherothrombotic process leading to acute arterial occlusion, which is manifest clinically by sudden death, myocardial infarction, stroke, and critical limb ischaemia. Work in our laboratory has already established that the structure and function of this fibrin clot is of consequence in relation to vascular disease and that insulin resistance with type 2 diabetes is associated with abnormalities of clot structure, although the cause of these findings in the setting of multiple metabolic abnormalities is unclear. We have just completed a BHF funded study looking at fibrin structure/function in pre-menopausal women with polycystic ovary syndrome to determine the effect of insulin resistance independent of glycaemia, and the role of post translational modifications to fibrinogen.

Representative publications
Scott EM, Åiens RAS, Grant PJ. Genetic and environmental determinants of fibrin structure and function: relevance to clinical disease. Arteriosclerosis, Thrombosis and Vascular Biology 2004; 24:1558-66.

Funding:
BHF, MCRC, Fund for International Research Collaborations, The Charitable Foundation

Current collaborators include Prof A Balen (Leeds), Prof B Staels (France), Prof U Smith (Sweden), Prof N Marx (Germany), Prof F Kuipers (Netherlands), Prof G Van Dijk (Netherlands), Prof J Takahashi (USA), Dr S Lockley (USA).

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Representative publications
Scott EM, Åiens RAS, Grant PJ. Genetic and environmental determinants of fibrin structure and function: relevance to clinical disease. Arteriosclerosis, Thrombosis and Vascular Biology 2004; 24:1558-66.
Abdominal Aortic Aneurysms

Abdominal Aortic Aneurysms (AAA) is an increasing problem in the ageing community. In the UK over 3,500 AAA operations are performed on an annual basis. They tend to affect elderly men in their seventies. As the AAA expands, clot is laid down inside the blood vessel. This traps white blood cells which then release various enzymes which breakdown the wall, leading to further expansion and rupture. Several studies have demonstrated that surgery should be reserved for those with an AAA which measures more than 5.5cm in diameter. It is at this point that the risk of rupture outweighs the operative risk of 5%. In this group of patients, repair is by the open route or by a minimal invasive technique called Endovascular aneurysms repair (EVAR). Both techniques use a graft or a covered metal stent to bypass the dilated segment of aorta. Despite the apparent success of these treatments, the vast majority of patients die from their associated heart disease.

The Leeds Aneurysm Development Study (LEADS) is based in the LiGHT and is funded by the Health Service Organisation. The long term aim is to recruit 1000 patients with Abdominal Aortic Aneurysms and a similar number of controls. The study focuses upon the relationship between inflammation and thrombosis and in particular the causes of AAA, and factors which affect expansion and the increased cardiovascular mortality within the AAA group.

In the short term we have funded a research nurse to collect blood samples from patients and controls to look at the haemostatic variables before and after intervention and to relate these findings with the presence of intraluminal thrombus. The study is supported by a Walport Clinical Lecturer Mr T Rashid and Intercalating BSc medical students.

Funding:
British Heart Foundation

More information:
http://www.leeds.ac.uk/medhealth/light/staff/scott_j.html

Representative publications
