

**60th Annual Meeting
British Microcirculation Society**

**Featuring a symposium on 'Diabetic Microangiopathy –
Old Players and New Contenders'**

19th – 20th April 2010

**Peninsula College of Medicine and Dentistry
Universities of Exeter and Plymouth**

**60th British Microcirculation Society Meeting
19th – 20th April 2010, followed by a Young Investigator's
Workshop on 21st April 2010
Peninsula Medical School, Exeter**

Foreword

Dear Participants,

It is my great pleasure to welcome you to the Peninsula Medical School, Exeter on behalf of the British Microcirculation Society and the Local Organising Committee.

This is the 60th Annual Meeting of the Society and we hope that you will enjoy the full and exciting scientific programme. The meeting includes a symposium on *Diabetic Microangiopathy – New Players and Old Contenders* which features distinguished speakers from the USA, Europe and the UK. We hope that this will highlight current research in this important area of the microcirculation. Of equal importance are the nineteen free oral communications and the poster presentations selected from a very high standard of abstract submissions.

The scientific meeting is followed by a 'Young Investigator' Workshop on 'How to get published' which is organised in collaboration with Wiley-Blackwell and will focus on good practice in writing research articles for peer-reviewed journals.

We would like to thank our many sponsors for their support and encourage you all to visit the trade exhibitors who have significantly contributed to the success of this meeting.

I hope you enjoy the meeting's social events as an opportunity to enhance scientific networking. It only remains for me to thank all of the delegates and invited speakers from local, national and overseas institutes for attending this exciting meeting. I wish you all a scientifically rewarding and enjoyable meeting.

Yours sincerely,

Jackie Whatmore

60th British Microcirculation Society Meeting
Peninsula Medical School, Exeter

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60th British Microcirculation Society Meeting Peninsula Medical School, Exeter

General Information

Meeting Venue: St Luke's Campus, University of Exeter, Magdalen Road, Exeter, EX1 2LU.

Registration: The Sports Hall, 09.00 – 16.00 each day.

Commercial Exhibition: The Sports Hall. Please note that stiletto heels should be avoided to prevent damage to the gym floor.

Oral Presentations: NC12 Lecture Theatre, North Cloisters. Power Point presentations should preferably be brought on a USB memory stick and loaded on the meeting projection PC at the start of the day of the presentation. Personal laptops are discouraged, but will be accommodated if necessary (e.g. if movies are shown or specialist software is required). Please contact Dr Jackie Whatmore if you intend to use your own laptop. Oral communications should be 10 minutes in length allowing 5 minutes for questions.

Poster Presentations: The Sports Hall. Boards are 1m wide and 2m high. Posters should be mounted by 10.30 on Monday 19th and be displayed for the whole meeting duration. Poster presenters will be advised when they are required to be at their posters. A panel of judges will select posters for the PromoCell, Moor and BMS poster prizes. Lunch and refreshments will be available during poster sessions.

Refreshments and Lunch: Coffee/teas and a finger buffet lunch will be included in the registration and served in the Sports Hall.

Accommodation: This is available on site (please book through the 2010 meeting website (<http://sites.pcmd.ac.uk/microsoc>) and in local hotels. A list of hotels can be found on the website. Luggage can be left securely in the Peninsula Medical School during the meeting.

Travel: Information on the meeting venue, maps and instructions are included at the back of this booklet and on the 2010 meeting website.

British Microcirculation Society Annual General Meeting: The AGM will be held at 17.15 – 18.30 in NC12 Lecture Theatre on Monday 19th April.

Drinks Reception and Gala Dinner at the Langstone Cliff Hotel, Dawlish Warren: Dinner tickets should be pre-booked at a cost of £45 per person which includes a drinks reception, 3 course meal, wine and disco. Coaches will depart from St Luke's at 18.45.

60th British Microcirculation Society Meeting
Peninsula Medical School, Exeter

Meeting Sponsors and Trade Exhibitors

This meeting is generously supported by

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Please take time to visit our commercial exhibitors.

Further information on these sponsors and links to their company webpages can be found on the meeting webpage at <http://sites.pcmd.ac.uk/microsoc/sponsors.php>

Monday 19th April

- 9.00 - Registration and Poster set-up – coffee/tea
- 10.50 – 11.00 Welcome address (Dr J Whatmore)
Introduction of Professor Sir John Tooke
(Professor G Clough)
- 11.00 – 11.30 **PromoCell Sponsored BMS Clinical Lecture
Professor Sir John Tooke
Diabetic Microangiopathy: Progress and
Puzzles**

SESSION 1 – ORAL COMMUNICATIONS

- OR1** 11.30 – 11.45 **A Salmon; University of Bristol**
VEGF_{165b} reduces albuminuria and reverses
increased glomerular permeability in animal
models of diabetic nephropathy
- OR2** 11.45 – 12.00 **G Mangialardi; University of Bristol**
Endothelial dysfunction in diabetic bone marrow
- OR3** 12.00 – 12.15 **X Cheng; Kings College London**
Impaired regulation of glutathione and the
NRF2 pathway in fetal endothelial cells from
diabetic pregnancies
- OR4** 12.15 – 12.30 **D Brazil; University College Dublin**
Mice lacking one allele of gremlin are protected
from diabetes-induced microvascular damage
in kidney
- OR5** 12.30 – 12.45 **M Meloni; University of Bristol**
AAV-2-mediated NGF gene transfer prevents
the development of heart microangiopathy and
cardiomyopathy in diabetic mice
- 12.45 – 14.45 **Lunch with poster and exhibition viewing**

SESSION 2: SPECIALISED SYMPOSIUM – PART 1

DIABETIC MICROANGIOPATHY – OLD PLAYERS AND NEW CONTENDERS

- S1** 14.45 – 15.15 **Luigi Gnudi; Kings College London, London, UK**
Haemodynamic factors in diabetic microvascular disease.
- S2** 15.15 – 15.45 **Alan Stitt; Queen’s University of Belfast, Belfast, UK**
Is glucose really the key player in diabetic microvascular complications?
- 15.45 – 16.15 **Tea**

SESSION 3: SPECIALISED SYMPOSIUM – PART 1 continued

DIABETIC MICROANGIOPATHY – OLD PLAYERS AND NEW CONTENDERS

- S3** 16.15 – 16.45 **Rayaz Malik; University of Manchester, Manchester, UK**
Microvascular structure in diabetes and obesity.
- S4** 16.45 – 17.15 **Anthony Adamis; Genentech, San Francisco, USA**
Is diabetic retinopathy an inflammatory disease?
- 17.15 - 18.30 **BMS Annual General Meeting**
- 18.45 **Coaches depart for reception and Society Dinner, Langstone Cliff Hotel, Dawlish Warren**

Tuesday 20th April

SESSION 4 – ORAL COMMUNICATIONS

- OR6** 8.30 – 8.45 **M Seager; University of Bristol, UK**
VEGF_{165b} overexpression inhibits ovarian follicle development via inhibition of angiogenesis
- OR7** 8.45 - 9.00 **S Bate; University of Sheffield, UK**
During sepsis does MAT.ang-1 protect the endothelium via eNOS?
- OR8** 9.00 – 9.15 **A Nicolson; University Hospital, Birmingham, UK**
Reperfusion as a marker of success of distal revascularisation
- OR9** 9.15 – 9.30 **C McVicar; Queen's University Belfast, UK**
Differential modulation of angiogenesis by erythropoiesis-stimulating agents in ischaemic retinopathy
- OR10** 9.30 – 9.45 **SC Satchell; University of Bristol, UK**
Effects of laminar flow on barrier properties of human glomerular endothelial cells
- OR11** 9.45 – 10.00 **I Packham; University of Birmingham, UK**
Differential role of platelets in physiological angiogenesis
- OR12** 10.00 – 10.15 **M Whiteman; Peninsula Medical School, UK**
Plasma hydrogen sulfide is a novel determinant of microvascular and macrovascular function: effect of adiposity
- 10.15 – 10.45 **Coffee**

SESSION 5 - ORAL COMMUNICATIONS

- OR13** 10.45 – 11.00 **U Reem; University of Sheffield, UK, University of Manchester, UK and Texas A&M University, USA**
Lipolysaccharide alters the function of rat mesenteric lymphatic vessels
- OR14** 11.00 – 11.15 **Z Gharaei; University of Sheffield, UK and University of Technology, Dresden, Germany.**
Cardiac endothelial cell damage by ionizing radiation
- OR15** 11.15 – 11.30 **D Kavanagh; University of Birmingham, UK**
Hematopoietic stem cells (HSCs) are recruited to injured gut by site specific adhesive mechanisms - recruitment can be enhanced
- OR16** 11.30 – 11.45 **R White; University of Birmingham, UK**
Modulating adhesion of hematopoietic stem cells to ischemia-reperfusion (IR) injured kidney sections
- OR17** 11.45 – 12.00 **C Staton; University of Sheffield, UK**
Expression of class 3 semaphorins and their receptors in human ductal breast disease
- OR18** 12.00 – 12.15 **Y-H Tang, University of Ulster, UK**
The role of the scaffold protein cybr in leukocyte recruitment during inflammation
- OR19** 12.15 – 12.30 **K Arkill; University of Bristol**
Fibre spacing in luminal endothelial glycocalyx
- 12.30 – 14.30 **Lunch with poster and exhibition viewing**

SESSION 6: SPECIALISED SYMPOSIUM – PART 2

DIABETIC MICROANGIOPATHY – OLD PLAYERS AND NEW CONTENDERS

- S5** 14.30 – 15.00 **Max Nieuwdorp: Academic Medical Center, Amsterdam, The Netherlands**
Dissecting the endothelial glycocalyx to minimize vascular risk profiles
- S6** 15.00 – 15.30 **Majid Kalani: Karolinska Institute, Stockholm, Sweden**
Vasoconstrictors – a major role in diabetic microangiopathy
- 15.30 – 16.00 **Tea**

SESSION 7: SPECIALISED SYMPOSIUM – PART 2 continued

DIABETIC MICROANGIOPATHY – OLD PLAYERS AND NEW CONTENDERS

- S7** 16.00 – 16.30 **Etto Eringa: University Medical Center, Amsterdam, The Netherlands**
Perivascular adipose tissue as a regulator of microvascular function and insulin sensitivity
- S8** 16.30 –17.00 **David Strain: Peninsula Medical School, Exeter, UK**
Microcirculatory abnormalities predict diabetes mellitus and end organ damage
- 17.00 **Close of meeting and presentation of poster prizes**

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SPECIALISED SYMPOSIUM

***DIABETIC MICROANGIOPATHY –
OLD PLAYERS AND NEW CONTENDERS***

[S1]

HAEMODYNAMIC FACTORS IN DIABETIC MICROVASCULAR DISEASE

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The epidemic of type 2 diabetes and the parallel rising incidence in end-stage renal disease is progressively increasing world-wide. Hypertension (increase glomerular pressure) and poor metabolic control seem to interact in causing the relentless decline in renal function seen in diabetic patients. It has been suggested that mechanical forces at the glomerular level may aggravate the metabolic insult by stimulating excessive cellular glucose uptake. We propose the existence of a self-maintaining cellular mechanism whereby a haemodynamic stimulus on glomerular cells (e.g. mesangial cells) induces greater glucose uptake and activation of intracellular metabolic pathways resulting in excess transforming growth factor β 1 (TGF β 1) and Vascular endothelial growth factor-A (VEGF-A) production. TGF β 1, one of the major pro-sclerotic cytokines in diabetic kidney disease, maintains the upregulation of cellular glucose uptake, and activates cellular events that result in increased extracellular matrix synthesis, podocytes apoptosis, podocyte altered cell adhesion and detachment, and formation of denuded areas on the glomerular basement membrane with alteration in vascular permeability. Changes in VEGF-A expression are closely related to altered vascular permeability, and its balanced expression appears to be very important in the normal function of the glomerular filtration barrier. VEGF-A and TGF β 1 represent two of the most important mediators of the haemodynamic-mediated disruption of the glomerular filtration barrier. Mechanical and metabolic coupling could represent an important mechanism of injury in the diabetic kidney.

[S2]

IS GLUCOSE REALLY THE KEY PLAYER IN DIABETIC MICROVASCULAR COMPLICATIONS?

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It is established that control of hyperglycaemia, dyslipidaemia and blood pressure can prevent the progression of diabetic microvascular complications such as retinopathy. However, it remains uncertain how these metabolic upsets mediate damage to microvascular cells at a cellular and molecular level. This lecture will review how glucose and lipids may contribute to microvascular dysfunction in the context of the diabetes. Using diabetic retinopathy as an exemplar microvascular complication, ongoing research into high glucose and lipid-mediated pathology will be presented. Complementary *in vitro* and *in vivo* studies will be used to illustrate glucose and lipid-linked pathogenic responses within the retina, especially relating to irreversible protein modification by advanced glycation endproducts (AGEs) and advanced lipoxidation endproducts (ALEs). The consequences of abnormal metabolism in terms of AGE/ALE formation will be discussed. In particular, our current knowledge about how AGE adducts influence retinal function in diabetes will be highlighted. There will also be emphasis placed on useful new therapeutic approaches to inhibit AGE/ALE formation or harmful receptor interactions to prevent key aspects of diabetic retinopathy such as acellular capillary formation, infiltration and activation of microglia and pro-inflammatory responses. Overall, the role of hyperglycaemia, the pathogenic pathways it evokes and the link to diabetic microvascular complications will be assessed.

[S3]

MICROVASCULAR STRUCTURE IN DIABETES AND OBESITY

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Vascular morphology and function were studied through the use of pressure myography to determine vascular morphology, mechanics, and myogenic responsiveness, together with testing of constrictor and dilator function. Small arteries from patients with essential hypertension (EH) demonstrated eutrophic inward remodelling and an increased distensibility. Vessels from type 2 diabetic patients demonstrated hypertrophy, a further increase in distensibility, and a highly significant loss of myogenic responsiveness compared with patients with EH and control patients. These results demonstrate vascular hypertrophy in small arteries from patients with type 2D as a consequence of impaired myogenic responsiveness which increases wall stress providing the stimulus for vascular hypertrophy. Studies in T1 D show that at baseline (BL) gluteal-fat small arteries from patients with systolic BP: 119 \pm 3 mm Hg; (n=12) had normal-resistance artery structure but patients with elevated systolic BP: 152 \pm 5 mm Hg; (n=5) demonstrate vascular hypertrophy. 10 years later (FU) 8 patients with improved cholesterol (FU: 3.9 \pm 0.2 mmol/l BL 4.9 \pm 0.2 mmol/l; P=0.01) and HbA1c (FU: 7.9 \pm 0.3% v BL: 8.9 \pm 0.6%; P=0.17), but increased systolic BP: FU: 136 \pm 3 mm Hg v BL: 119 \pm 6 mm Hg; P=0.03) demonstrated eutrophic remodelling. These findings suggest that, with poor metabolic control, small arteries from patients with type 1 DM show hypertrophy in response to elevated BP, which is comparable to T2DM. However, metabolic improvements enable eutrophic remodelling to occur in response to an increase in BP.

[S4]

IS DIABETIC RETINOPATHY AN INFLAMMATORY DISEASE?

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Animal models demonstrate an accumulation of inflammatory cells in the retinal vasculature shortly after the onset of experimental diabetes. The inflammatory cells utilize CD18 and ICAM-1 to adhere to the vasculature and appear to be operative in pathogenesis of many of the signature pathologies of diabetic retinopathy - breakdown of the blood retinal barrier, platelet microthrombi, pericyte loss, microaneurysms and acellular capillary formation. Human correlates are being identified and the accumulated data suggest that diabetic retinopathy is a chronic low grade inflammatory disease.

[S5]

DISSECTING THE ENDOTHELIAL GLYCOCALYX TO MINIMIZE VASCULAR RISK PROFILES

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The endothelial glycocalyx has emerged as a potential orchestrator of vascular homeostasis. Under physiological conditions, the glycocalyx is an important contributor to the regulation of vascular permeability, uptake of macromolecules (including cholesterol) as well for the adhesion of circulating cells. In line, the potential role of the glycocalyx in maintaining the anti-atherogenic properties of the vessel wall may have important clinical implications. Thus, novel reliable methods to estimate glycocalyx dimensions *in vivo* as well as progressive insight into the enzymes involved in glycocalyx synthesis will be crucial in the assessment of this structure as a potential surrogate marker or therapeutic target against both micro and macrovascular disease. An overview will be provided on endothelial glycocalyx heparan sulphates in health and disease. Moreover, preliminary data regarding vascular function and glycocalyx in endothelial heparansulphate knockout mice will be presented followed by a glance at the future of establishing endothelial glycocalyx as a crucial player and therapeutic target in human (micro)vascular disease.

[S6]

VASOCONSTRICTORS – A MAJOR ROLE IN DIABETIC MICROANGIOPATHY

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Most of the late diabetic complications have their basis in disturbed microvascular function. Structural and functional changes in the microcirculation are present in diabetes mellitus irrespective of the organ studied, and the pathogenesis is complex. Endothelial dysfunction, characterized by an imbalance between endothelium-derived vasodilator and vasoconstrictor substances, plays an important role in the pathogenesis of diabetic microangiopathy. Increased circulating levels of vasoconstrictors, e.g. endothelin-1 (ET-1), has been found in patients with diabetes, and a positive correlation between plasma ET-1 levels and microangiopathy in patients with type 2 diabetes has been demonstrated. In addition to its direct vasoconstrictor effects, enhanced levels of ET-1 may contribute to endothelial dysfunction through inhibitory effects on nitric oxide (NO) production. Vascular endothelial dysfunction may precede insulin resistance, although the feature of insulin resistance syndrome includes factors that have negative effects on endothelial function. Furthermore, ET-1 induces a reduction in insulin sensitivity and may take part in the development of the metabolic syndrome. The mechanisms by which ET-1 contributes to the development of diabetic microangiopathy and the potentially beneficial effect of ET_A receptor antagonists are discussed.

[S7]

PERIVASCULAR ADIPOSE TISSUE AS A REGULATOR OF MICROVASCULAR FUNCTION AND INSULIN SENSITIVITY

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Obesity, i.e. accumulation of adipose tissue, is associated with insulin resistance and cardiovascular disease. Microvascular dysfunction may contribute to insulin resistance and hypertension in obesity by reducing muscle perfusion and increasing vascular resistance. We have hypothesized that microvascular dysfunction in obesity is caused by perivascular adipose tissue (Yudkin, Eringa et al., *The Lancet* **365**, 1817-1820). Indeed, perivascular adipose tissue in the heart and around the aorta has been found by several groups to produce a variety of vasoactive substances, such as cytokines and adiponectin. In muscles of db/db mice and diet-induced obese mice, we have found accumulation of perivascular adipose tissue (mPAT). In functional studies, mPAT of lean mice induced insulin-mediated vasodilatation of muscle resistance arteries, an effect lost in obese db/db mice. This vasoregulatory effect of mPAT was accompanied by secretion of adiponectin, which was decreased in mPAT of db/db mice. Blockade of adiponectin action with a soluble fragment of the adiponectin R1 receptor abolished the interaction between mPAT and insulin-mediated vasoreactivity, whereas addition of globular adiponectin mimicked the mPAT effect. In summary, perivascular adipose tissue in a variety of vascular beds produces vasoactive substances. In muscle, perivascular adipose tissue controls insulin-mediated vasodilatation by secretion of adiponectin, and this function is impaired in experimental obesity. These findings shed new light on the pathogenesis of vascular dysfunction and insulin resistance in obesity.

[S8]

MICROCIRCULATORY ABNORMALITIES PREDICT DIABETES MELLITUS AND END ORGAN DAMAGE.

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As the proportion of older adults increases across the developed world, a greater understanding of the aetiopathogenic mechanisms of the increased vascular risk associated with ageing and their therapeutic implications becomes essential. The endothelium and microcirculation are integral to the maintenance of adequate perfusion and their dysfunction is thought to be an early and potentially reversible mechanism to increase cardiovascular risk. Ageing is associated with impaired microvessel endothelial function and an increase in capillary blood pressure, independent of brachial artery blood pressure. Biological and lifestyle factors that influence microvessel function include body fat and visceral adiposity, sex hormone status, diet and physical activity. Abnormalities of the microcirculation have been reported in a number of disease states including hypertension, insulin resistance and type I & II diabetes. Elevated urinary albumin excretion rate (AER), a clinically recognised surrogate of systemic microvascular dysfunction, independently predicts total and cardiovascular mortality in both diabetic and non-diabetic subjects. Similarly, retinopathy is associated with a 3-fold increase of heart failure. Cerebral microvascular disease predicts stroke and if retinopathy co-exists the risk ratio rises from 2.6 to 18.1, suggesting greater risk is conferred by systemic microvascular dysfunction. Exploration of the therapeutic implications for management of endothelial dysfunction remains in embryonic state, however benefit in terms of reduced cardiovascular damage, proportionate to improvements in AER have been demonstrated.

SELECTED ORAL COMMUNICATIONS

[OR1]

VEGF_{165b} REDUCES ALBUMINURIA AND REVERSES INCREASED GLOMERULAR PERMEABILITY IN ANIMAL MODELS OF DIABETIC NEPHROPATHY

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Albuminuria occurs early in diabetic nephropathy (DN). A recent hypothesis proposes that “the onset of albuminuria is not associated with changes in glomerular capillary wall permeability”^[1]. We measured glomerular permeability coefficients directly shortly after the onset of proteinuria in two animal models of streptozotocin (STZ)-induced diabetes. We also tested the effect of VEGF_{165b} on albuminuria and glomerular permeability in these models of DN. Mice expressing human VEGF-A_{165b} in podocytes under control of the nephrin promoter (nephVEGF-A_{165b}), or their wild-type littermates (WT), were injected with 100mg/kg STZ daily for 3 days to induce diabetes; Sprague-Dawley rats received a single injection of 45mg/kg body weight STZ; sham-treated animals received citrate buffer alone. Glomerular water permeability ($L_P A/V_i$) was measured oncometrically^[2] in single isolated glomeruli *ex vivo*. The diabetes-induced increases in albuminuria (5.4-fold: $p < 0.05$ vs sham) and $L_P A/V_i$ (1.4-fold: $p < 0.05$ vs sham) in WT mice were both reduced by VEGF_{165b} overexpression. $L_P A/V_i$ was also increased (1.7-fold: $p < 0.05$ vs sham) in proteinuric diabetic rats; this increase was reversed by incubating glomeruli in 1nM VEGF_{165b} for 1 hour ($p < 0.05$). Glomerular permeability increases early in animal models of DN. VEGF_{165b} reverses these permeability changes and reduces albuminuria. 1] Russo et al. (2009) *J Am Soc Nephrol* **20**:489-494. [2] Salmon et al. (2006) *J Physiol* **570**:141-156

[OR2]

ENDOTHELIAL DYSFUNCTION IN DIABETIC BONE MARROW

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OBJECTIVE: Diabetes mellitus contributes to the progression of endothelial dysfunction in a variety of tissues but little is known about the bone marrow endothelium. Here we demonstrate that *ex vivo* cultured bone marrow endothelial cells (BMEC) from streptozotocin-induced type 1 diabetic mice show typical features of endothelial dysfunction. METHODS AND RESULTS: BMEC were isolated under selective culture conditions and depleted of myeloid/monocytic BM cells. Purity of the endothelial cell population was assessed by flow cytometry and immunocytochemistry. Cultured BMEC from diabetic mice showed higher levels of oxidative stress in mitochondria, increased activity of the senescence marker beta-galactosidase, reduced migratory capacity toward VEGF-A and SDF-1 and reduced network-formation capacities in a Matrigel assay. Freshly isolated bone marrow mononuclear cells (BM-MNC) showed an increased adhesiveness either under static conditions or under the influence of shear flow to diabetic endothelial layer. Furthermore, diabetic BMEC showed an enhanced permeability as demonstrated by a lower resistance in trans-endothelial resistance assay. This latter effect was reverted by N-Acetyl-cysteine (N-Ac). Spontaneous trans-endothelial migration of BM-MNCs in presence of diabetic endothelium was increased, whereas responsiveness to SDF-1 was abrogated. CONCLUSIONS: Taken together these results suggest that diabetes induces endothelial barrier dysfunction. These alterations may have profound consequences for the liberation of progenitor cells into the circulation and more in general for BM stem cell homeostasis

[OR3]

IMPAIRED REGULATION OF GLUTATHIONE AND THE NRF2 PATHWAY IN FETAL ENDOTHELIAL CELLS FROM DIABETIC PREGNANCIES

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Gestational diabetes is associated with a higher incidence of diabetes and cardiovascular diseases in offspring [1]. Previous studies demonstrated that fetal vascular function is altered in diabetic pregnancies, but the underlying mechanisms remain to be elucidated [2]. Reactive oxygen species (ROS) and play an important role in the development of diabetes and associated vascular dysfunction [3]. We hypothesised that a diabetic intrauterine environment (gestational diabetes) will affect fetal programming and epigenetic changes, leading to alterations antioxidant signalling. The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway is a key defence against oxidative stress and regulates glutathione levels. Human umbilical vein endothelial cells (HUVEC) from normal and diabetic pregnancies were cultured in 20% serum M199 and then treated with 4-hydroxynonenal (HNE) 20 μ M for 3-24h. Glutathione (GSH) and antioxidant enzyme levels were measured by fluorescence and immunoblotting, respectively. GSH and NAD(P)H:quinone oxidoreductase (NQO1) expression were increased in response to HNE in normal HUVEC but markedly diminished in HUVEC from gestational diabetic pregnancies. Our findings suggest that impaired antioxidant defences due to epigenetic changes may contribute to the development of diabetes in adulthood.

[1] BUCHANAN, T.A. ET AL. (2005) J. CLIN. INVEST. 115:485-491

[2] SOBREVIA, L. ET AL. (1995) J. PHYSIOL. 489:183-192

[3] THOMAS, S.R. ET AL. (2008) ANTIOX. REDOX. SIGNAL.10:1713-1765

[OR4]

MICE LACKING ONE ALLELE OF GREMLIN ARE PROTECTED FROM DIABETES-INDUCED MICROVASCULAR DAMAGE IN KIDNEY

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Gremlin is an antagonist of the bone morphogenetic protein family that plays a key role in limb bud development and kidney formation. There is a growing appreciation that altered gremlin expression may regulate the homeostatic constraints on damage responses in microvascular diseases such as diabetic nephropathy. Here we have explored whether knockout mice heterozygous for *grem1* gene deletion (*grem1*^{+/-}) exhibit protection from the progression of diabetic nephropathy in a streptozotocin-induced model of type 1 diabetes. A marked elevation in *grem1* expression was detected in the kidneys of diabetic wild-type mice compared to littermate controls, particularly in kidney tubules. In contrast, diabetic *grem1*^{+/-} mice displayed a significant attenuation in *grem1* expression at 6 months of diabetes compared to age and sex-matched wild-type controls. Whereas the onset and induction of diabetes was similar between *grem1*^{+/-} and wild-type mice, several indicators of diabetes-associated microvascular kidney damage such as increased glomerular basement membrane thickening and microalbuminuria were attenuated in *grem1*^{+/-} mice compared to wild-type controls. Markers of renal damage such as connective tissue growth factor were elevated in diabetic wild-type, but not *grem1*^{+/-} kidney. Levels of pSmad1/5/8 decreased in wild-type, but not *grem1*^{+/-} diabetic kidneys, suggesting that protective BMP signalling may be maintained in the absence of *grem1*. These data therefore identify *grem1* as a potential modifier of renal microvascular injury in the diabetic kidney.

[OR5]

AAV-2-MEDIATED NGF GENE TRANSFER PREVENTS THE DEVELOPMENT OF HEART MICROANGIOPATHY AND CARDIOMYOPATHY IN DIABETIC MICE

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Diabetes mellitus can cause cardiac dysfunction and heart failure. The neurotrophin nerve growth factor (NGF) promotes survival of both endothelial cells and cardiomyocytes, but it is downregulated in the diabetic heart. The present study challenged the hypothesis that NGF gene transfer (GT) could prevent diabetes-induced cardiac dysfunction. Streptozotocin-induced type 1 diabetic mice were injected in the left ventricle (LV) with an adeno-associated vector carrying human NGF (AAV-2-hNGF) or an empty vector (AAV-2- β Gal). Age-matched normoglycemic mice injected with AAV-2- β Gal served as controls. X-Gal staining confirmed successful GT of LV. Moreover, hNGF protein expression was detected in mouse plasma at 12 weeks. Echocardiography at 12 weeks after GT showed a deterioration of systolic function in diabetic mice indicated by reduced LV ejection fraction and fractional shortening. In contrast, AAV-2-hNGF improved both parameters compared to diabetic controls. Moreover, AAV-2-hNGF improved LV pressure and dP/dt_{max} and dP/dt_{min} (measured by Millar catheter). Analyses at 12 weeks indicated that diabetes induced microvessel apoptosis and rarefaction in the myocardium. By contrast, AAV-2-hNGF preserved capillary and small arteriole densities in diabetic mice. Also, as measured by fluorescent microspheres, diabetes reduced LV blood flow which was prevented by AAV-2-hNGF. These results provide evidence that prolonged NGF overexpression prevents systolic dysfunction and preserves cardiac microvasculature in diabetic mice

[OR6]

VEGF_{165b} OVEREXPRESSION INHIBITS OVARIAN FOLLICLE DEVELOPMENT VIA INHIBITION OF ANGIOGENESIS

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Ovarian follicle development requires VEGF mediated angiogenesis. VEGF pre-mRNA is differentially spliced to form two families, VEGF_{xxx} and VEGF_{xxx}b, where xxx denotes the amino acid number. VEGF_{165b} inhibits VEGF₁₆₅-induced angiogenesis, and is endogenously expressed in the ovary. To identify the ovarian function of VEGF_{165b}, we generated transgenic mice (TG) overexpressing human VEGF_{165b} under the control of the MMTV promoter. RT-PCR indicated expression of hVEGF_{165b} in ovaries of TG females. TG dams mated with WT male mice produced litters half the size of WT dams mated with TG or WT males. No difference in pup birth weight or developmental defects were identified in pups from TG mothers suggesting subfertility due to defects in the female reproductive system. The number of embryos released into oviducts in TG females was significantly lower than that in WT females (81% of WT), and they lacked surrounding cumulus cells, although in *in vitro* culture they survived to morula stage as well as those from WT females. There were fewer secondary (17.3±7.8) and tertiary (1±1) follicles in TG ovaries compared with WT (55.6±13.9 and 6±2.1) when 0.5 day-postcoitus ovaries were analysed and follicles had a smaller vascular area per unit perimeter (0.15±0.02 vs. 0.28±0.04 μm²/μm, p<0.01, N=12). This suggests that overexpression of VEGF_{165b} in the ovary may reduce fertility by inhibiting angiogenesis required for follicle development. Supported by the Wellcome Trust & BHF.

[OR7]

DURING SEPSIS DOES MAT.ANG-1 PROTECT THE ENDOTHELIUM VIA ENOS?

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During sepsis, an altered balance of iNOS and eNOS is associated with microvascular dysfunction[1]. Angiotensin-1 (Ang-1) is a peptide with vessel stabilising effects in mature vessels which activates eNOS via Tie-2 receptors on the endothelium[2]. MAT.Ang-1 is a variant of Ang-1 with higher solubility (>95% vs. 60-70%) and potency (95% vs. 75% Tie-2 receptor binding)[3]. We hypothesise that eNOS expression and activation will be reduced in a murine model of sepsis, but increased in septic animals treated with MAT.Ang-1. Abdominal striated muscle from C3H/HeN mice (total n=12) was used for Western blot analysis of eNOS (BD Biosciences) and phosphorylated eNOS (AbCam) at 24h with *E. coli* lipopolysaccharide (1mg/kg LPS (055:B5) i.p.) with and without MAT.Ang-1 (33ug i.v.) at 19h. The following groups were studied: (i) control, (ii) LPS, (iii) MAT.Ang-1 and (iv) LPS+MAT.Ang-1. eNOS expression was reduced in the LPS group (vs. control), but increased in the presence of LPS+MAT.Ang-1 (vs. control and LPS). eNOS expression was greatest with MAT.Ang-1 alone. The presence of phosphorylated eNOS was demonstrated in all samples and further analysis is underway. In conclusion, MAT.Ang-1 counteracts decreased expression of eNOS in septic mice. Thus protective effects of MAT.Ang-1 on the endothelium may be mediated through increased expression of eNOS. [1] Doursout et al. (2008). Shock 29(6):692-702. [2] Saharinen et al. (2008). Nat Cell Biol 10(5):527-37. [3] Cho et al. (2004). P Natl Acad Sci USA 101(15):5547-52.

[OR8]

REPERFUSION AS A MARKER OF SUCCESS OF DISTAL REVASCULARISATION

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In critical limb ischaemia (CLI) microvascular reactivity is attenuated resulting in loss of postural vasoconstriction (PVC) and impaired reactive hyperaemia (RH). Restoration of perfusion by surgical bypass (ABS) appears to normalize PVC and RH [1] but effects of transluminal angioplasty (PTA) are less clear. To compare these treatments, patients with CLI were assigned to ABS (n=15) or PTA (n=7). Tissue perfusion at the toe pulp was quantified before and 6 weeks after intervention using Laser Doppler fluxmetry to assess PCV (change from supine to sitting) and RH (post-2min arterial occlusion). Spectral analysis was performed on toe flux oscillations. Revascularisation was better after ABS than PTA (anatomical severity index decreased from 33 pre-intervention to 4 and 17 respectively). Toe perfusion pressure (50 ± 5 mmHg pre-intervention) also increased more after ABS than PTA (59 ± 12 , 27 ± 16 mmHg). PVC was absent pre-intervention since flux remained unchanged ($-7\pm 9\%$) with legs dependent and was unaltered by ABS or PTA. Pre-intervention RH was sluggish reaching a peak perfusion 20-40% above baseline only after 2min. With ABS and PTA, RH peak was attained after 40-50sec, reaching values 40-60% above baseline. Spectral analysis (FFT) of resting flux oscillations corresponding to myogenic, neural and endothelial components did not show any differences pre- to post-intervention. There thus appears little amelioration of impaired microvascular reactivity up to 6 weeks following revascularization by ABS or PTA, despite better symptomatic relief in the ABS group.

[1] Midttun et al. 1999. Eur J Vasc Endovasc Surg 17:225-229

[OR9]

DIFFERENTIAL MODULATION OF ANGIOGENESIS BY ERYTHROPOIESIS-STIMULATING AGENTS IN ISCHAEMIC RETINOPATHY

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Erythropoietin (EPO) regulates angiogenesis and erythropoietic stimulating agents (ESAs) may have beneficial properties for microvascular re-modelling following ischaemic injury. In the retina, ischaemia leads to neovascularisation and sight-threatening retinopathy and in the current study we have used three distinct ESAs which are in current clinical use to determine how they differentially regulate the retinal response to ischaemia *in vivo*. We used the murine model of oxygen induced retinopathy (OIR) and mice received 30 or 2500IU/Kg of epoetin delta, epoetin beta and equivalent doses of darbepoetin alfa or control. Only epoetin beta induced a dose-dependent pre-retinal neovascularisation ($p < 0.05$). However, epoetin delta induced a significant intra-retinal vessel forming response which reduced ischaemia ($p < 0.05$) and enhanced normality of the vasculature ($p < 0.05$). With epoetin delta-treatment this retinal vascularisation response occurred concomitantly with increased bone marrow mobilisation of haematopoietic stem cells and enhanced localisation of these cells to immature retinal vessels. darbepoetin alfa induced retinal cytokine mRNA expression of $TNF\alpha$ and VEGF, ($p < 0.001$). This data suggests that epoetin delta appears to promote revascularisation without impacting on pathological, pre-retinal neovascularisation. These findings have implications for ESA usage in anaemic patients who could have enhanced risk of tumour neovascularization and proliferative retinopathies.

[OR10]

EFFECTS OF LAMINAR FLOW ON BARRIER PROPERTIES OF HUMAN GLOMERULAR ENDOTHELIAL CELLS

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We have shown that endothelial Nitric Oxide Synthase (eNOS) phosphorylation and NO production is increased in human glomerular endothelial cells (hGEnCs) following exposure to physiologically relevant shear. We determined the effects of shear directly on hGEnCs barrier properties using the electric cell substrate impedance sensing (ECIS) flow module. The hGEnCs were grown in flow chambers, then exposed to intermittent shear, as follows; 30-minutes of shear at 10 dynes/cm² followed by a 4 hour period of no shear. After 24 hours, the experiment was paused and either 100µM of L-N^G-monomethyl Arginine citrate (L-NMMA, an NOS inhibitor) or vehicle added and the experiment re-started. Following a pre-incubation of 30 minutes, intermittent shear was initiated as detailed above. In all experiments, a flow chamber under no flow conditions was used as a non-shear control. Transendothelial electrical resistance TEER was recorded in real-time. A sharp yet modest reduction in the mean TEER was observed at the onset of flow relative to the no shear control (p<0.05, paired t-test). The TEER then started to recover before flow was removed and continued thereafter before reaching basal values. This transient reduction in hGEnCs TEER following the onset of shear was inhibited following treatment with 100µM of L-NMMA, but not the vehicle (p<0.01, paired t-test). Thus showing that NO has direct autocrine effects on hGEnC permeability and suggests the importance of eNOS and NO in regulation of glomerular filtration.

Supported by the BBRSC.

[OR11]

DIFFERENTIAL ROLE OF PLATELETS IN PHYSIOLOGICAL ANGIOGENESIS

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Platelet depletion (PD) inhibits tumour angiogenesis (capillary growth from pre-existing vessels), however a role in physiological angiogenesis is undetermined. We independently induced physiological capillary growth by longitudinal splitting or endothelial sprouting in C57/BL6 mice and determined a differential response to PD. Longitudinal splitting induced by the α_1 adrenoceptor antagonist prazosin resulted in a significant increase in capillary:fibre ratio (C:F), a measure of angiogenesis, in *m. extensor digitorum longus* at 7 days. PD by rat anti-mouse GPIIb α antibody did not affect the outcome, and had no effect on C:F of untreated mice. Endothelial sprouting induced by unilateral extirpation of *m. tibialis anterior* under isoflurane anaesthesia, causing synergistic muscle overload, induced a significant C:F increase compared to contralateral limbs. PD had no effect on contralateral limbs, but completely abolished the C:F increase seen in overload limbs. Pharmacological inhibition of platelet activity with clopidogrel hydrogensulfate/acetylsalicylic acid led to similar changes in C:F as PD. To help elucidate mechanisms, we repeated overload in mice with twofold greater expression of vascular endothelial growth factor (VEGF), a molecule with well-reported angiogenic properties, but saw no difference in C:F increase +/-PD despite a raised baseline C:F. This demonstrated that VEGF overexpression alone is unable to 'rescue' an angiogenic phenotype after platelet depletion.

Supported by the BHF.

[OR12]

PLASMA HYDROGEN SULFIDE IS A NOVEL DETERMINANT OF MICROVASCULAR AND MACROVASCULAR FUNCTION: EFFECT OF ADIPOSITY.

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Hydrogen sulfide (H₂S) is a recently identified endogenous endothelium-dependent vasodilator however its role in the human vasculature is not clear. Plasma was obtained from male patients with type II diabetes (T2DM;n=11), overweight (OW;n=16) and lean (n=11) volunteers. H₂S levels were determined by zinc trap spectrophotometry. Anthropometric measurements, blood biochemistry, systemic blood pressure and microvascular function (minimum vascular resistance, MVR and maximum hyperemia, MH) were determined. Median plasma H₂S levels in age matched lean, overweight and T2DM volunteers were 38.9 (29.7, 45.1) μmol/l, 22.0 (18.6, 26.7) μmol/l and 10.5 (4.8, 22.0) μmol/l respectively. Median plasma H₂S levels were significantly lower in T2DM patients compared to lean ($p=0.001$) and OW volunteers ($p=0.008$) and H₂S levels in OW were significantly lower than in lean controls ($p=0.003$). Plasma H₂S levels correlated with indices of microvascular (MVR, $-0.436, p=0.004$; MH, $+0.402, p=0.008$) and macrovascular function (SysBP, $-0.850, p<0.001$; DiastBP, $-0.527, p=0.001$). Central adiposity was an independent predictor of plasma H₂S ($R^2=0.423$, standardized beta: -0.650 , $P<0.001$). This relationship was independent of diabetes which only contributed a further 5% to the model ($R^2=0.477$). Plasma H₂S may be useful determinant of vascular health

[OR13]

LIPOLYSACCHARIDE ALTERS THE FUNCTION OF RAT MESENTERIC LYMPHATIC VESSELS

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Lymphatic vessels become damaged during sepsis leading to stagnation of lymphatic flow and tissue oedema. Angiopoietin-1 (Ang-1) is a growth hormone that regulates vascular permeability via Tie-2 [1]. However, its effects on lymphatics are unknown. Mesenteric collecting lymphatics (~80µm) were dissected from Male Sprague Dawley rats and mounted on a pressurised myograph system at 1-5cmH₂O. Images were recorded onto DVD and changes in diameter, along with the frequency and amplitude of spontaneous contractions [2], were measured in response to lipopolysaccharide (055:B5, 50µg/ml) added to the organ bath to mimic sepsis. A considerably increased frequency, decreased amplitude of contractions with no changes in diameter was observed at 1.5h progressing to inhibition of pumping activity at 3h. A stable and active variant of Ang-1, MAT.Ang-1 [3] (250ng/ml, intraluminally) caused a marked increase in diameter and frequency, but no change in amplitude after 1.5 and 3 h. Fluorescent confocal microscopy of isolated lymphatics, using an anti-mouse Tie-2 polyclonal goat IgG antibody incubated with an Alexa-Fluor anti-goat IgG confirmed that rat mesenteric lymphatic vessels express Tie-2. Studies are underway to determine whether MAT.Ang-1 can improve lymphatic function during sepsis.

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[OR14]

CARDIAC ENDOTHELIAL CELL DAMAGE BY IONIZING RADIATION

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Epidemiological studies suggest that even moderate to low radiation doses to the heart can increase the risk of cardiovascular disease that sometimes manifests decades after exposure. Microvascular damage could play a crucial role in the pathogenesis of radiation-induced heart disease. Cardiac injury triggers angiogenesis-dependent repair mechanisms in the myocardium. Here, we investigated how ionizing radiation affects endothelial integrity and angiogenic responses in the mouse heart. The hearts of C57BL/6 mice were locally irradiated with doses of 0.2, 2, 8 or 16 Gy and animals sacrificed 20 weeks post-irradiation. Angiogenesis was assessed in heart explants embedded in fibrin gels. Formation of angiogenic sprouts was scored at 10 days and confirmed by lectin staining. Radiation inhibited sprout formation dose-dependently, with significant changes observed with 8 and 16 Gy. The angiogenic capacity of irradiated hearts was also assessed in a novel fibroblast-endothelial self-assembling assay we developed. Hearts were enzymatically dissociated into single cells and cultured for 10 days. Endothelial cells migrated, proliferated and remodelled into capillary-like structures among fibroblasts and pericytes. Radiation inhibited the viability of extracted cells and the number and quality of capillary tubes. Our data show that radiation causes persistent vascular damage and inhibits angiogenic activity in the heart, which could influence the development of heart disease.

[OR15]

HEMATOPOEITIC STEM CELLS (HSCs) ARE RECRUITED TO INJURED GUT BY SITE SPECIFIC ADHESIVE MECHANISMS - RECRUITMENT CAN BE ENHANCED

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Introduction: HSCs can be recruited to injury sites and subsequently aid tissue repair. Mechanisms governing recruitment following injury are poorly understood and it is not known whether these are site specific. We therefore utilized intravital microscopy to elucidate mechanisms involved in HSC (HPC-7) recruitment to gut and cremaster muscle microcirculation following ischemia-reperfusion (IR) injury.

Methods: The ileum or cremaster of anaesthetised (ketamine/xylazine) mice was subjected to ischemia. IgG, anti-CD18 or anti-CD49d pre-treated HPC-7s were introduced during reperfusion and their adhesion monitored. Some HPC-7s were incubated (20 min) with homogenised IR injured gut conditioned media (IR-CM) prior to determining their adhesion to frozen IR injured gut sections *in vitro*. **Results:** IR injury significantly enhanced HPC-7 adhesion in ileum ($p < 0.05$) and cremaster ($p < 0.05$) compared to controls. Adhesion was significantly reduced by anti-CD18 ($p < 0.05$) pre-treatment in ileum whereas anti-CD18 ($p < 0.05$) and anti-CD49d ($p < 0.01$) reduced adhesion in cremaster. Incubation of cells with IR-CM significantly ($p < 0.05$) increased their adhesion to gut sections compared to naïve cells.

Conclusion: We have previously demonstrated that CD49d is critical for hepatic HSC recruitment. Collectively, these studies suggest site specific homing mechanisms govern HSC recruitment to injured tissue. Furthermore, as yet unidentified factors released during injury can enhance HSC adhesion – this has important therapeutic implications.

Supported by the BHF

[OR16]

MODULATING ADHESION OF HEMATOPOIETIC STEM CELLS TO ISCHEMIA-REPERFUSION (IR) INJURED KIDNEY SECTIONS

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Introduction: Experimental studies demonstrate HSCs migrate to ischemically injured kidney and aid in tissue repair. However, clinical success is poor, partially due to limited HSC recruitment. Numerous factors may influence recruitment including (i) duration of injury (ii) pre-treatment of HSCs with potential homing factors released from injured sites. This study assessed adhesion of naïve and pre-treated HSCs (HPC-7s) to frozen sections of IR injured kidney *in vitro*. **Methods:** The left kidney of anaesthetised (ketamine/xylazine) mice was subjected to ischemia followed by 1 or 2 hrs reperfusion. HPC-7 adhesion to sham, IR injured or contralateral (CL) kidney sections was determined. Some HPC-7s were incubated for 5 or 10 min with media conditioned with homogenised IR kidney (IR-CM). **Results:** Significant adhesion of HPC-7s to IR ($p < 0.01$) and CL ($p < 0.05$) kidney was observed after 1 hr rep when compared to shams. Adhesion was significantly greater following 2 hr rep in both IR ($p < 0.05$) and CL ($p < 0.01$) kidney compared to adhesion at 1 hr. HPC-7 pre-treatment with IR-CM for 5 min induced significant adhesion to both sham ($p < 0.05$) and IR injured kidney ($p < 0.001$) compared to non-treated cells. 10 min ICM pre-treatment further increased HPC7 ($p < 0.001$) compared to 5 min. **Conclusion:** HSC adhesion following renal injury is dependent upon severity of injury. Furthermore, as yet unidentified factors released during renal injury can also enhance adhesion. Approaches that improve renal homing may improve the clinical outcome of stem cell therapies.

[OR17]

EXPRESSION OF CLASS 3 SEMAPHORINS AND THEIR RECEPTORS IN HUMAN DUCTAL BREAST DISEASE

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Class 3 semaphorins (sema3) are soluble ligands that inhibit VEGF activity by competing with VEGF for binding neuropilins (Np1&2) and have been shown to be down regulated during disease progression in ovarian cancer. Therefore this study aimed to test the hypothesis that Sema3A expressions are also down-regulated during disease progression in breast cancer. Immunohistochemistry of human breast specimens (n=176) including normal human breast, benign and pre-malignant hyperplastic tissue, pre-invasive and invasive breast cancer revealed that weak Sema3A and Sema3F expression was present in normal breast epithelium and significantly decreased with lesion severity ($p < 0.014$). In contrast sema3B expression was strong in normal breast epithelium and only decreased with the transition from pre-invasive to invasive cancer ($p = 0.001$). Significant inverse correlations were seen between VEGF and Sema3B expression (Spearman's $\rho = -0.289$; $p = 0.008$). Receptor analysis revealed that Np2 was expressed in 90% of epithelial/tumour cells and 80% endothelial cells and does not change with lesion severity. In contrast Np1 expression in both epithelial and endothelial cells significantly increased with increasing lesion severity to pre-invasive cancer ($p < 0.035$). Work is currently underway to assess Np1 expression in invasive cancer. These data suggest that a decrease in class 3 semaphorin and increase in VEGF expression may be associated with disease progression in ductal breast carcinoma.

Supported by Yorkshire Cancer Research

[OR18]

THE ROLE OF THE SCAFFOLD PROTEIN CYBR IN LEUKOCYTE RECRUITMENT DURING INFLAMMATION

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Cytohesin Binder and Regulator (Cybr) is a scaffold protein highly expressed in the hematopoietic/immune system whose physiological role is poorly understood. *In vitro* studies indicate that Cybr expression regulates LFA-1, a crucial molecule in lymphocyte attachment and migration through the endothelial monolayer. Cybr also binds cytohesin-1, a guanine nucleotide exchange factor for the ARF GTPases, which affects actin cytoskeleton remodelling during the process of cell migration [1]. Here we show the distribution of (the different sub-classes of) leukocytes in immune organs of Cybr-null (Cybr^{-/-}) in comparison to wild type (WT) mice under normal and experimentally induced inflammatory conditions. Cybr^{-/-} reduces lymphocyte number in peripheral lymph nodes ($1.95 \pm 0.31 \times 10^6$ versus WT $3.47 \pm 0.57 \times 10^6$ cells, $p < 0.05$) and Peyer's patch ($5.0 \pm 0.63 \times 10^5$ versus WT $10.2 \pm 2.18 \times 10^5$ cells, $p < 0.05$). The number of leukocytes in peripheral blood of Cybr^{-/-} mice is $6.48 \pm 0.57 \times 10^9/L$ compared to WT $6.81 \pm 1.02 \times 10^9/L$, (NS: $p > 0.05$). However on day 3 following induction of aseptic peritonitis (4% thioglycolate i.p.:n=8 for each group) Cybr^{-/-} mice have significantly greater numbers of leukocytes in the bloodstream than wild-type littermates $6.65 \pm 1.98 \times 10^9/L$ versus WT $4.36 \pm 1.38 \times 10^9/L$, ($p < 0.05$), respectively. Cybr^{-/-} mice had a similar number of peritoneal inflammatory cells compared with the wild type controls ($26.76 \pm 6.89 \times 10^6$ versus WT $29.71 \pm 8.45 \times 10^6$). This data is consistent with the hypothesis that the characteristic intense extravasatory response of inflammatory cells from the peripheral circulation in wild-type mice is impeded in Cybr^{-/-} littermates where a more sustained period of leukocyte migration is observed. This hypothesis is currently being examined by counting leukocyte extravasation (rolling, adhesion and extravasation) in Cybr^{-/-} mice using the dorsal skinfold window chamber model [2] and a variety of experimental conditions involving induction of inflammation (addition of inflammatory mediators, injury and tumour growth).

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[2] Guerreiro-Lucas et al. (2008) 76:161-168

[OR19]

FIBRE SPACING IN LUMINAL ENDOTHELIAL GLYCOCALYX

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Although the endothelial cell glycocalyx (ECG) is thought to determine the permeability of microvascular walls to macromolecules, surprisingly few data define the structural basis for such filtering [1]. In this study we have used transmission electron microscopy (TEM) micrographs from rat organs and tissues (including: kidney, stomach (corpus fundus), and retina) fixed according to the Rostgaard perfusion technique [2] to improve preservation of ECG. The ECG appeared in two distinct forms: tufts, usually above fenestrations; or an even lining over the whole membrane. Using a perfected autocorrelation protocol, the global fibre spacing parallel to the ECG membrane was found to be 28nm, with an estimated fibre diameter of 20nm, leaving a 'void' of 8nm. This should exclude, if the fibres are negatively charged, serum albumin (mol.radius=3.6nm) [3]. Model fibre structures were used to validate the 2D technique and enable calibration of a conversion into 3D. TEM tomograms were also used to find the longer spacings between the tufts of ECG. The primary outcome is a tested protocol which can be used on any electron micrographs of ECG to give reliable structural parameters which will be invaluable in analysing future permeability experiments.

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POSTER PRESENTATIONS

VASCULAR CELL ADHESION AND ADHESION MOLECULES

[PP1]

A NEUTRALISING ANTIBODY FOR VEGF-A_{165b} PROMOTES THE PRO-MIGRATORY EFFECT OF VEGF₁₆₅ *IN VITRO* AND IS CYTOTOXIC TO RPE CELLS.

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Anti-angiogenic therapies in cancer and ocular disease that target vascular endothelial growth factor-A (VEGF-A) inhibit the action of all VEGF-A isoforms, although systemic toxicities to high-dose anti-VEGF-A therapies are emerging. VEGF-A₁₆₅ has pro-migratory effects *in vitro* and *in vivo* and has been identified as a key factor in angiogenic-dependent diseases. VEGF-A_{165b} arises from alternative splicing of exon 8 and crucially is anti-angiogenic and has cytoprotective properties. To determine if VEGF-A_{165b} antibodies were neutralising, human umbilical vein endothelial cells (HUVECs) were migrated toward a chemoattractant, 1 nM VEGF-A₁₆₅, in transwell migration assay. 0.3 nM VEGF-A_{165b} significantly ($p < 0.0001$) reduced migration by $36 \pm 4.68\%$ and a monoclonal antibody specific for VEGF_{165b}, 56/1 (12 ng/ul) inhibited the anti-migratory effect of VEGF-A_{165b} by $24 \pm 4.96\%$ having no significant effect compared to VEGF₁₆₅ administration ($p > 0.05$). Treatment of human retinal pigmented epithelial cells (ARPE-19) with 1 mg/ml 56/1 increased cytotoxicity by $120 \pm 34\%$ as measured by LDH-release cytotoxicity assay, indicating an endogenous role for VEGF-A_{165b} in retinal epithelial cell survival. This evidence for VEGF-A_{165b} as an endogenous anti-migratory and cytoprotective factor strongly support VEGF-A_{165b} as an attractive alternative therapeutic to anti-VEGF-A therapy in numerous cancers and ocular disease.

[PP2]

VEGF OVER-EXPRESSION INCREASES GLOMERULAR WATER PERMEABILITY *IN VIVO* IN A CONDITIONAL AND INDUCIBLE MOUSE MODEL

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VEGF increases glomerular permeability to water ($L_P A/V_i$) *in vitro* on isolated intact glomeruli [1]. We further investigated the role of VEGF on $L_P A/V_i$ when over-expressed *in vivo* in a mouse model. A double-transgenic mouse model of kidney-specific inducible VEGF expression was generated (Pod-rtTA x tetO-VEGF₁₆₄). Double-transgenic mice or wild-type controls were given doxycycline (2mg/ml) for 1, 3, 7 days or 14 weeks. Spot urine collection was used to quantify proteinuria. Renal cortex was harvested for measurement of VEGF expression, and the remaining tissue was used in permeability studies using the method described in [1]. There was no significant difference upon 1 day of induction (TG, Mean±SEM $L_P A/V_i$ = 0.93±0.20 WT 0.82±0.17). At 3 days post-induction $L_P A/V_i$ increased 2.05-fold from 0.71±0.07 in WT to 1.47±0.24 in TG and at 7 days 2.49-fold from 0.78±0.12 in WT to 1.94±0.26 in TG. VEGF cortical levels and proteinuria were not significantly different at 1 day and 3 days post-induction. After 7 days treatment with doxycycline, VEGF levels were increased 2.55±0.42 fold and proteinuria 1.52±0.12-fold. Following long induction for 14 weeks, no further increase in proteinuria was observed and $L_P A/V_i$ values, though higher in the TG group (2.7±0.58) compared with WT (1.52±0.24), were not significantly different (p=0.09, N=10).

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[PP3]

EFFECT OF OVEREXPRESSION OF VEGF_{165b} IN MOUSE SALIVARY ON DYE PERFUSION

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Vascular Endothelial Growth Factor (VEGF₁₆₅) increases permeability and is a vasodilator, whereas the anti-angiogenic splice form of VEGF_{165b} inhibits VEGF₁₆₅ mediated endothelial cell proliferation, migration, vasodilatation and in vivo angiogenesis, and only transiently increases hydraulic conductivity. Transgenic (TG) mice were generated over-expressing VEGF_{165b} under the control of the mouse mammary tumour virus promoter. In male adult TG mice, the VEGF_{165b} transgene was expressed only in salivary gland and lung, but not in other organs screened. 100 µl of Evans' blue (E-2129, Sigma) was perfused into 5 male TG mice (30 µg/g), and 5 wild type male littermates, and 20 min later mice were sacrificed and salivary glands extracted. VEGF_{165b} expression was increased in salivary gland as evidenced by RT-PCR for the human cDNA. There was a small but significant increase in Evans blue dye in the salivary tissues (162.6±8.4 vs. 135.6±5.1 µg/g, n=5, p<0.05). These results indicate that VEGF_{165b} over-expression in the salivary gland affected the vasculature, although whether by altering angiogenesis, permeability, perfusion, capillary number or other haemodynamic parameter is unclear.

[PP4]

NEUROPILIN EXPRESSION IN THE NORMAL COLON RELATES TO BUTYRATE LEVELS

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Fermentation of fibre in the colon leads to production of short chain fatty acids including butyrate. Neuropilin-1 (Np1) is a transmembrane receptor for VEGF and semaphorins and is a known transcriptional target of Sp1 whose activity is regulated by butyrate. Previous studies have suggested that Np1 expression is limited within normal tissues of the gastrointestinal tract and we hypothesise that butyrate may regulate the expression of Np1 in the colon. Biopsies of normal colon were taken from 26 patients at the mid-sigmoid and stained for Np1 and Chromogranin-A (CGA; enteroendocrine cell marker). Stool samples were collected and analysed for butyrate levels, which were correlated with Np1 and CGA expression. Butyrate within the stool samples ranged from 0.6-16mM. NRP-1 was found to be expressed in a subset of cells in the normal colon epithelium, representing between 0 and 3% of the cells in the crypt. A strong inverse correlation was seen between faecal butyrate levels and the percentage of NRP-1 positive cells per crypt (Spearman's rho = -0.622; p=0.0001) and between faecal butyrate levels and the percentage of CGA positive cells per crypt (Spearman's rho = -0.370; p=0.053). Co-localisation analysis revealed that only 11% of Np1 expressing cells were also positive for CGA. These data suggest that Np1 is butyrate responsive in the colon suggesting it has a role in normal colon epithelial function, but the majority is not expressed in enteroendocrine cells.

Supported by Yorkshire Cancer Research.

[PP5]

EFFECTS OF GLUCOSE ON EXPRESSION OF VEGF 165A/VEGFB SPLICE VARIANTS IN HUMAN NORMAL AND DIABETIC PLACENTAL MICROVESSELS.

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The ratio of the splice variants VEGF165a (pro-angiogenic; pro-permeability) and VEGF165b (anti-angiogenic; pro-permeability) may influence vascular development and function. Their expression in normal and Type 1 diabetic human placenta and their vulnerability to hyperglycaemic insult is not known. Chorionic villous explants from normal (N) and Type 1 diabetic (D) study groups were incubated in 5mM or 15mM glucose for 4h (N5, D5; N15, D15). VEGF-165a/VEGF-165b was immunolocalised and vascular counts were obtained. D5 explants contained significantly lower numbers of VEGF165b positive vascular profiles compared to N5. In normal explants, 15mM glucose induced a decrease ($p < 0.01$) in vessels expressing VEGF165b; the values were now similar to D5. This effect was not seen in the D15 which remained similar to that of D5 and N15. VEGF165a staining, which was higher in diabetic euglycaemic explants, showed increased expression at 15mM glucose in both normal and diabetic groups ($p < 0.001$). There was a negative correlation between VEGF165a and VEGF165b staining ($p < 0.001$; $R^2 = 0.6852$). Thus glucose can affect the expression of both splice variants of VEGF. High glucose can induce the diabetic phenotype in normal explants, i.e. an upregulation of VEGF165a and downregulation of 165b. In diabetics, hyperglycaemia further altered this phenotype with increases in the pro-angiogenic VEGF whilst expression of the splice variant 165b remains damped. The ratio of the two VEGF splice variants may be an important predictor of vascular dysfunction in diabetes. *Funded by ASGBI.*

TUMOUR MICROCIRCULATION

[PP6]

OVARIAN CANCER CELLS INDUCE INCREASED PROLIFERATION AND TUBE STRUCTURE FORMATION IN HUMAN OMENTAL MICROVASCULAR ENDOTHELIAL CELLS.

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Background: Ovarian cancer frequently metastasizes to the omentum. Although the molecular mechanisms of metastatic invasion are not understood, it is likely that angiogenesis is induced in the activated omental microvasculature i.e. endothelial cell proliferation and new vessel formation. A knowledge of the specific interactions between ovarian cancer cells and human omental microvascular endothelial cells (HOMECS) is critical for understanding the spread of ovarian cancer. Aim: To investigate the effects of factors released from ovarian cancer cells (SKOV3 cells) on the proliferation and angiogenic tube like formation of HOMECS. Methods: The proliferation of HOMECS incubated in SKOV3 conditioned medium (CM) was assessed by a colourimetric assay. An in vitro tubule formation assay was performed to investigate the angiogenic potential of SKOV3 cells. Results: CM significantly increased proliferation of HOMECS to 116.54±23.72%, 144.89±60.52% and 240.17±193.16% vs. control (100%) ($p < 0.001$, $n = 54$) at 24, 48 and 72 hours respectively. In co-culture SKOV3 cells induced tube structure formation in HOMECS, as did VEGF (100ng/ml). VEGF-induced tube structure formation was significantly inhibited by SU5416 (a VEGF receptor inhibitor), whereas SKOV3-induced tubule formation was not inhibited. Conclusion: SKOV3 cells secrete potent factors that increase proliferation and angiogenic tube structure formation in SKOV3 cells. Initial results suggest that SKOV3-induced angiogenesis may not be VEGF dependent.

[PP7]

SUPPRESSION OF TUMOUR GROWTH IN MICE BY OVER-EXPRESSION OF TIA-1 IN HUMAN COLON CANCER CELLS

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We have previously reported that T cell intercellular antigen 1 (TIA-1), an RNA binding protein, is alternatively spliced in adenocarcinoma colon cells and that over-expression of the full length TIA-1 resulted in change in the expression of the vascular endothelial growth factor (VEGF) isoforms. In this study we investigated whether over-expression of TIA-1 may alter tumor growth in relation to VEGF isoforms expression. TIA-1 transfection in two colon cancer cells (LS174t and SB/10C), harboring spliced TIA-1, were found to up-regulate the anti-angiogenic isoform of VEGF, VEGF_{165b}. To determine whether this would block tumour growth by inhibiting angiogenesis, 2×10^6 cells LS174t expressing TIA-1 cells, or vector transfected cells were injected into nude mice. At 21 ($382 \pm 60 \text{mm}^3$) and 25 ($866 \pm 217 \text{mm}^3$) days post-injection of 2×10^6 cells, tumor growth was significantly reduced compared to control tumors (1190 ± 267 and 2117 ± 551 respectively, both $p < 0.05$, One way Anova). Tumour vascularization (vessels/ mm^2) in TIA-1 transfected tumors was significantly reduced (1.8 ± 0.35 compared with 3.6 ± 0.4 vector, 4.9 ± 0.4 control, $p < 0.05$). We conclude that TIA-1 by regulating splicing of VEGF to favour anti-angiogenic isoforms may inhibit angiogenesis in colon cancer.

[PP8]

PERFUSION MONITORING DURING REAL-TIME DERMATOLOGICAL METHYL-AMINOLEVULINATE PHOTODYNAMIC THERAPY (MAL-PDT)

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Perfusion was monitored with a laser Doppler perfusion imager (LDPI) in real-time within a range of (pre)cancerous skin lesions (actinic keratosis, Bowen's disease and superficial basal cell carcinomas) undergoing initial and subsequent methyl-aminolevulinate photodynamic therapy (MAL-PDT) treatments. The successful ablation of cancerous tissue during MAL-PDT relies on three factors: a photosensitiser (protoporphyrin IX, PpIX) being formed from the pro-drug, MAL, light of the appropriate wavelength and oxygen. Perfusion was monitored for the first time, before, during and after light irradiation in MAL-PDT to determine whether lesional blood flow altered. The initial perfusion was significantly greater ($P<0.01$) within (pre)cancerous lesions than distal normal skin and significant variation ($P<0.05$) was observed between histologically distinct lesions. Perfusion within lesions altered during light irradiation, with the overall trend indicating an increase ($P<0.05$) in perfusion after half the light treatment. The perfusion was then reduced ($P<0.05$) following the completion of light delivery. Subsequent treatment cycles resulted in similar changes in perfusion but perfusion was consistently lower ($P<0.05$) within lesions during the second treatment cycle. The data collected permits greater understanding of perfusion alterations and thus potential oxygen availability within the localised tissue. Understanding these complex physiological changes during MAL-PDT potentially enables derivation of optimum treatment parameters.

[PP9]

CHARACTERISATION OF TWO MALIGNANT ENDOTHELIAL CELL LINES AND COMPARISON OF CHEMOTHERAPY RESPONSE

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Angiosarcomas (AS) are rare malignant vascular tumours. We have obtained two human cutaneous AS cell lines (ASM and ISO-HAS) and are currently investigating their endothelial properties and response to chemotherapeutic agents known to interact with the vasculature. The cell lines have been characterised using a panel of endothelial cell markers and compared to normal human dermal microvascular endothelial cells (HuDMECs). ASM and ISO-HAS demonstrated expression of CD31, CD34 and vWF, and over expressed VEGFA and VEGFR2 relative to HuDMEC controls. ASM cell proliferation was assessed in response to Paclitaxel (P) and Doxorubicin (D) chemotherapy and the tyrosine kinase inhibitor Axitinib (A). 10^5 ASM cells/ml/well were seeded in 12 well plates and allowed to establish. Chemotherapy was introduced at 48 hours and cell counts performed at 96 and 120 hours. Concentrations of 0, 1, 5, 10, 25, and 50ng/ml P and D were compared. At 96 hours IC_{50} were 12ng/ml and 23ng/ml for D and P respectively. At 120 hours IC_{50} were 6.5ng/ml and 8ng/ml respectively. The IC_{50} for single agent A was 125ng/ml. Combinations of P (1ng and 5ng/ml) and A (0- 100ng/ml) were assessed, with combination therapy more potent than either single agent alone. IC_{50} was achieved with P 5ng/ml and A 10ng/ml. Further experiments to explore endothelial chemoresponsiveness are underway as both AS cell-lines demonstrate endothelial cell characteristics.

[PP10]

KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS INFECTION ENHANCES ENDOTHELIAL CELL MOTILITY

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Extensive aberrant angiogenesis is a hallmark of Kaposi's sarcoma (KS), a complex endothelial cell (EC) tumour. Kaposi's sarcoma-associated herpesvirus (KSHV)-infected spindle cells line the abnormal vascular spaces. This distribution suggests a role for KSHV in aberrant angiogenesis. Indeed, KSHV is recognized as the causal agent of KS, but the mechanism is incompletely understood. Angiogenesis requires EC migration. To investigate the impact of KSHV upon EC motility, human umbilical vein EC were infected with KSHV and cultured for 1 to 10 days before being transferred to either 8µm pore transwell filters or a wound closure assay. KSHV-infected cultures more efficiently transmigrated across the transwell filters. The infected cells transmigrated preferentially compared to uninfected cells, which migrated equivalent to controls. These data suggest either a direct mechanism of enhanced motility or a soluble mediator acts selectively upon infected cells. Infection also increased in-plane migration, accelerating wound closure up to 7 days post infection. Initial studies indicate KSHV may modify matrix deposition and integrin subunit expression; both would be relevant to motility and vessel growth *in vivo*. Besides angiogenesis, increased EC motility might facilitate other facets of KS pathogenesis, such as infected cell dissemination, recruitment from the microcirculation and virus persistence. Understanding the mechanisms of KSHV enhancement of EC motility might identify novel ways to intervene in the lifecycle and pathogenesis of the virus.

[PP11]

ESCAPE FROM BREAST CANCER THERAPY, ARE NEUROPILINS THE KEY?

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Vascular Endothelial Growth Factor (VEGF) correlates with disease progression and poor prognosis in breast cancer. Bevacizumab (Bz), an anti-VEGF antibody, inhibits VEGF binding to VEGF-R1/2 and increases progression free survival in breast cancer patients. Eventually tumours escape treatment control and we hypothesise that Bz does not prevent VEGF from binding to its alternative receptors, the neuropilins (Np1 & Np2). PCR revealed that VEGF and Np1 are expressed in human dermal microvascular endothelial cells (HuDMECS) and three breast cancer cell lines (MCF7, MDA-MB-231 & MDA-MB-436). Analysis of tubule formation of HuDMECs revealed that three Np1 binding peptides (P2, P7b & P10) significantly inhibited tubule formation when measured as number of tubules or branch points ($p < 0.005$), whereas a fourth peptide (P1) had no effect. In contrast none of these peptides had a significant effect on HuDMEC or breast cancer cell proliferation in the MTS assay, although P10 caused a slight inhibition of MDA-MB-436 proliferation. In contrast Bz demonstrated a profound inhibition of HuDMEC tubule formation and a small inhibition in proliferation. Work is currently underway to assess the effects of Bz on breast cancer cell proliferation. These data show that both endothelial and breast cancer cells express Np1, and one Np1 blocking peptide (P10) inhibits both endothelial cell differentiation and breast cancer cell proliferation, suggesting that P10 may have therapeutic potential for breast cancer treatment.

Supported by Breast Cancer Campaign

[PP12]

FACTORS RELEASED BY A549 AND SK-MES LUNG TUMOUR CELL LINES INCREASE THEIR ADHESION TO HUMAN CEREBRAL MICROVASCULAR CELLS

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Background: Lung tumours frequently metastasise to the brain. During cerebral metastasis formation the tumour cells must adhere to, and migrate through, the endothelial cell (EC) layer that lines the cerebral blood vessels. We have previously shown that factors released from lung tumour cell lines increase adhesion molecule expression on cerebral ECs. Aim: To examine whether factors released from lung tumour cells alter their adhesion to cerebral ECs. Methods: Two different lung tumour cell lines A549 (adenocarcinoma) and SK-MES (squamous cell carcinoma) were cultured in a defined basal medium (DMEM-BS) and the factors released by the tumour cells were collected (conditioned medium (CM)). Adhesion of lung tumour cells to human cerebral microvascular endothelial cells (hCMEC-D3) was assessed using an *in vitro* flow assay. hCMEC-D3 cells were seeded on flow chambers and treated with CM or DMEM-BS for 4hrs or 24hrs. The hCMEC-D3 cells were then exposed to the relevant lung tumour cells under flow conditions. The results were quantified by phase contrast microscopy. Results: Compared to control levels (100%) SK-MES CM significantly increased cell adhesion after 4hrs and 24hrs to $1029.2 \pm 247.1\%$ ($p=0.037$, $n=3$) and $399.5 \pm 140.4\%$ ($p=0.005$, $n=5$) respectively. A549 CM also significantly increased cell adhesion after 4hrs and 24hrs to $299.9 \pm 41.6\%$ ($p=0.037$, $n=3$) and $347.5 \pm 193.6\%$ respectively ($p=0.014$, $n=5$). Conclusions: Proteins/ factors released from lung tumour cell lines increase tumour cell adhesion to cerebral ECs.

[PP13]

CHRONIC SPHINGOSINE-1-PHOSPHATE (S1P) TREATMENT OF MICE WITH TUMOURS EXPRESSING VEGF120 ENHANCES TUMOUR GROWTH RATE

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Mouse fibrosarcomas expressing a single vascular endothelial growth factor (VEGF)-A isoform (120) under endogenous promoter control have been developed. Subcutaneous VEGF120 tumours transplanted into mice are highly vascularised, with poor vessel (pericyte deficient) development, and are susceptible to vessel breakdown by tubulin-binding vascular disrupting agents (VDAs). Disruption of adheren junctions is a putative primary mode of action for VDAs in vivo. Perivascular mural cells in tumours contribute to the integrity of adheren junctions, conferring resistance to disruption. S1P can increase VE-cadherin associated junction integrity. Here, we determined the effect of chronic treatment of VEGF120 tumour-bearing mice with S1P1 on tumour growth and vascularisation (VE-cadherin and N-cadherin expression, vascular density, pericyte investment, vascular volume), and tumour necrosis. We also determined if S1P treatment conferred resistance to the VDA, combretastatin (CA-4-P). We found S1P treatment enhanced tumour growth rate, and by day 7, treated tumour volumes were 17% greater than saline treated tumours. These results implicate the importance of adheren associated junctions for vascular stability and tumour growth, and their potential for vascular targeting. Further insight into the role of S1P in the development of the tumour microcirculation should provide new leads for vascular targeting and anti-cancer therapy.

STEM / PROGENITOR CELLS

[PP14]

EARLY ENDOTHELIAL PROGENITOR CELLS ACT AS PRO-ANGIOGENIC MYELOID CELLS

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Endothelial Progenitor Cells (EPCs) have been shown to facilitate revascularisation of ischaemic tissues and hold therapeutic promise for promoting repair and reperfusion in conditions such as acute myocardial infarction, stroke and diabetic microvasculopathies. Two distinct EPC cell populations, early EPCs (eEPCs) and Outgrowth endothelial cells (OECs), have been isolated *in vitro*. Although OECs are considered the EPC type with more progenitor features, evidence indicating a pro-angiogenic role for eEPCs cannot be disregarded. This study investigates the eEPC phenotype and their angiogenic properties. eEPCs exhibited minimal proliferative potential. FACS analysis indicated high expression of haematopoietic markers CD45 and CD14. This was confirmed at the transcriptome level by high expression of haematopoietic transcripts (HLAs, LYZ, TLRs, CD163 and CD14). eEPCs were compared to monocytes and endothelial cells using genome-wide transcriptomics, 2D protein profiles, and electron microscopy. The three approaches indicated eEPCs closely resemble monocytes. Although eEPCs have no intrinsic tubulogenic potential *in vitro*, they significantly promote endothelial tube formation in co-cultures ($p < 0.05$). In conclusion, our evidence indicates eEPC phenotype is closer to monocytes than endothelial cells. Therefore, eEPCs actually represent a pro-angiogenic myeloid cell.

[PP15]

TRANSCRIPTOMICS STUDY OF THE SENESCENT PROGRAM IN ENDOTHELIAL PROGENITOR CELLS

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Endothelial Progenitor Cells (EPCs) play a key role in vascular homeostasis. Cell therapies based on delivering EPCs to ischaemic tissues face the difficulty of generating sufficient cell numbers. Adult peripheral blood-derived EPCs have significant expansion capacity; however they lose replicative potential after 20-34 population doublings. This study investigates EPC senescence program using a genome-wide transcriptome analysis. EPC senescence is characterised by a significant increase in cytoplasmic volume in unison with β -Galactosidase activity ($p < 0.01$); loss of cell proliferation as demonstrated by decreased BrdU incorporation ($p < 0.01$); DNA damage accumulation shown by a significant increase in γ -H2AX positive cells; and decrease of telomerase activity. Transcriptome analysis comparing pre-senescent with senescent EPCs identified 828 overexpressed and 705 underexpressed transcripts (FDR=0.05 and fold-change threshold=1). Interestingly, upregulated transcripts can be classified into three main groups: Interferon signalling pathway-associated transcripts (IFI27, MX1, IFI6, OAS2, GAS6); transforming growth factor- β signalling pathway-related transcripts (TGFB1, TGFB2, FAP, Follistatin-like 3, COL4A1); and secreted inflammatory cytokines (CXCL6, IL8, CCL5, IL1B, CCL2). In conclusion, EPCs have a limited replicative potential and undergo senescence with gene expression changes in specific molecular pathways. This study suggests that modulating these molecular pathways might allow control of EPC senescence rate.

[PP16]

BI-DIRECTIONAL MIGRATION OF MESENCHYMAL STEM CELLS ACROSS BONE MARROW ENDOTHELIAL CELLS

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In this study we used an in vitro model to determine the migratory cues that drive MSC migration in a basal-to-apical (bone marrow to circulation) and apical-to-basal (circulation to bone marrow) direction across human bone marrow endothelial cells (HBMECs). MSCs were imaged on the apical surface of HBMECs with or without a chemokine. Results showed that MSCs did migrate across the HBMECs with the percent of migrated cells increasing from 15% to 65% with CXCL12 ($p < 0.001$). In a second method HBMECs were grown in a transwell system. The total number of MSCs that transmigrated and adhered to the underside of the filter were counted. Five chemokines all increased the number of migrated cells with CXCL16 having the biggest influence increasing from 100 cells in the control up to 600. Both of these methods were models of MSC migration in the apical-to-basal direction. The transwell method was then altered to look at MSC migration in the basal-to-apical direction. To do this the HBMECs were grown on the underside of the filter. Again all five chemokines significantly increased the number of migrated MSCs, the largest increase being from 100 to approximately 200 ($p < 0.01$) with CXCL16. These results show that chemokines stimulate MSC migration across HBMECs in both directions. Thus these chemokines may enhance recruitment of MSCs from the circulation to the bone marrow and mobilisation of MSCs into the circulation.

REGULATION OF VASCULAR TONE AND BLOOD FLOW AND REPERFUSION

[PP17]

THE EFFECT OF BLOOD FLUX ON MEAN BLOOD SATURATION (S_{mbO_2}) IN THE CUTANEOUS MICROCIRCULATION

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Optical reflectance spectroscopy (ORS) and near infrared spectroscopy measure the mean blood saturation (S_{mbO_2}) in the microcirculation as changes in concentration of oxyhaemoglobin [HbO_2] and deoxyhaemoglobin [Hb]. For S_{mbO_2} to be a clinical useful tool it is essential that we understand when a reduction in S_{mbO_2} indicates potential hypoxia to the tissue. It has been shown that S_{mbO_2} is dependent not only on oxygen uptake by tissue but is also altered by spontaneous fluctuations in blood volume arising from the effects of respiration, endothelial, sympathetic and myogenic activity [1]. This study aims to establish the role of blood flux as determined by laser Doppler fluximetry (LDF), on the measurement of S_{mbO_2} . Simultaneous measurements by ORS and LDF were made at the same site in the microcirculation of dorsal forearm skin of 24 healthy males (age 25-68 years). In 18 out of 24 subjects, Fourier analysis identified periodic fluctuations in S_{mbO_2} resulting from a fall in [HbO_2] accompanied by a rise in [Hb], with little change in blood volume. Blood flux remained relatively constant as [HbO_2] fell and [Hb] rose but a sudden transient surge in flux then increased S_{mbO_2} . The frequencies of oscillation were $<0.02\text{Hz}$ and are attributed to the effects of endothelial activity. This suggests that the rise in S_{mbO_2} represents increased arterial inflow whilst the fall in S_{mbO_2} may indicate oxygen uptake by the tissue.

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[PP18]

THE RESPONSE OF HUMAN PODOCYTES TO PERFUSION PRESSURE IS DIFFERENT TO RAT PODOCYTES

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In rat and rabbit kidney the position of the podocyte cell body relative to underlying glomerular filtration barrier (GFB) was shown to be a close one (1,2). The sub podocyte space (SPS) thus formed was shown to be sensitive to perfusion pressure with the SPS height after colloid perfusion, being lower than that during fixation with no perfusion pressure ($0.34 \pm 0.1 \mu\text{m}$ v. $0.53 \pm 0.03 \mu\text{m}$) suggesting the podocyte was responding to ultrafiltration pressure. The response with human podocytes and SPS was expected to be similar. With ethical committee approval, a transplant kidney rejected from the procedure because of damage was sampled for immersion fixed specimens and fixed in buffered glutaraldehyde. The sampled area was clamped and the rest of the kidney perfused with oncologically balanced Mammalian Ringer and then a similarly balanced buffered glutaraldehyde. Tissues were processed for electron microscopy using standard protocols. Measurements of human podocytes showed a pressure response opposite to rat podocytes with the SPS height being greatest ($0.48 \pm 0.06 \mu\text{m}$) in perfused human glomeruli (~ 30 mmHg ultrafiltration pressure) and lower ($0.30 \pm 0.04 \mu\text{m}$) in immersion fixed glomeruli (0 mm Hg ultrafiltration pressure). Human podocytes have a pressure response opposite to the podocytes of experimental animals, the solutions used for human kidney transport may have affected the podocyte response.

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[PP19]

INFLUENCE OF HYPERCHOLESTEROLEMIA ON SPREADING DILATATION RESPONSES IN MOUSE MESENTERIC ARTERIES

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In mouse mesenteric arteries, the PAR2 ligand SLIGRL (30 μ M) evokes robust endothelium-dependent hyperpolarization (EDH) and full dilatation. In addition to local dilatation, the endothelium forms the conduit for distal spread of hyperpolarizing signals along the longitudinal axis of resistance arteries, resulting in spreading dilatation. We isolated and triple-cannulated mouse mesenteric arteries in a gravity-fed pressure myograph at 37°C, with one branch of the bifurcation connected to a syringe pump containing SLIGRL and a fluorescent dye. Constant downstream flow through the feed artery allowed luminal perfusion of agonists into the sidebranch, and using confocal microscopy we simultaneously measured local dilatation in the sidebranch and the spreading dilatation into the feed artery, the latter measured in 500 μ m intervals upstream from the bifurcation. The EDH-type dilatation to SLIGRL was significantly augmented in young (9-14 week old) mice. In both cases spreading dilatation was also observed, but when normalized to 80% dilatation at 0 μ m, there was no difference between the groups, both with similar mechanical length constants near 2 mm. In contrast, the spreading dilatation to SLIGRL was markedly reduced in older ApoE^{-/-} mice, reducing the length constant by more than 50%. Small lipid deposition was observed by oil red O staining in the aortas from young ApoE^{-/-} mice, which was markedly enhanced in the older ApoE^{-/-} animals. This was paralleled by expression of Mac-3 in macrophage-rich areas within the same atherosclerotic lesions. These findings suggest that in small mesenteric arteries of ApoE^{-/-} mice, the EDH-type dilatation is paradoxically enhanced in the early stages of vascular disease, with reduced ability for intercellular coupling and spreading dilatation as disease progresses.

[PP20]

ACTIVATION OF β -ADRENOCEPTORS EVOKES LOCAL AND SPREADING DILATATION IN RAT ISOLATED MESENTERIC ARTERIES

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Adrenaline and noradrenaline (NA) change arterial tone by their action on different adrenoceptors. We investigated if adrenergic agonists can evoke conducted vasomotor response in rat pressurized small mesenteric arteries, and which receptor subtypes are involved. Both adrenaline and NA applied intraluminally in mesenteric arteries evoked concentration-dependent vasoconstriction. Adrenaline was more potent than NA ($\log EC_{50} = -6.60 \pm 0.07$ vs. $\log EC_{50} = -5.89 \pm 0.05$). Application of phentolamine ($1 \mu\text{M}$) inhibited vasoconstriction and uncovered concentration-dependent vasodilatation in U46619-precontracted arteries. In triple-cannulated arteries, local application of isoprenaline ($1 \mu\text{M}$) led to spreading dilatation against the direction of intraluminal flow, a response similar to the effect of the endothelium-dependent agonist acetylcholine. Adrenaline ($1 \mu\text{M}$) did not evoke spread of contraction; however, in the presence of prazosin ($1 \mu\text{M}$) both adrenaline ($1 \mu\text{M}$) and NA ($1 \mu\text{M}$) caused spreading dilatation. Yohimbine ($1 \mu\text{M}$) enhanced both adrenaline and NA-mediated local dilation, but did not alter the extent of spreading vasodilatation. Propranolol ($1 \mu\text{M}$) attenuated, and together with SR59230A ($1 \mu\text{M}$) abolished both local and spreading responses. Overall, this study indicates β -adrenoceptor-mediated spread of vasodilatation over significant distances in resistance arteries. This suggests an important role for β -adrenoceptors in microcirculation.

[PP21]

A PILOT STUDY INVESTIGATING MYOGENIC ACTIVITY DURING RECOVERY FROM LOCAL HEATING IN HEALTHY SUBJECTS

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Maximum skin hyperaemia (MH) induced by locally heating to $\geq 42^{\circ}\text{C}$ is a well established method of investigating microvascular function. An area of interest is the recovery of skin hyperaemia following a thermal challenge. Previous research demonstrated that this recovery response involves nitric oxide. This study aims to evaluate the involvement of myogenic activity, assessed by spectral analysis, on skin hyperaemic recovery following local heating. Skin hyperaemia of the ventral forearm was continuously monitored by single point laser Doppler fluximetry at rest (10mins), during local heating to 42°C (30mins) and during post-heating recovery (20mins) in 6 healthy subjects. Using Fourier analysis, peak spectral amplitude was calculated for frequency interval representing myogenic activity (0.05-0.15Hz) for baseline, MH (last 5 mins of heating) and for post-heating recovery (within first 10 mins of recovery). There was a significant difference in myogenic activity across the three phases with a four fold increase in myogenic activity during heating and a 12 fold increase during recovery (peak amplitude: rest: median: 1.65(25th, 75th Centiles 0.83, 2.37), heating: 7.49(5.80, 13.19), recovery: 20.49(11.83, 24.17) $p=0.002$ Friedmans test). Myogenic activity was significantly higher during post heating recovery compared to rest and heating ($p=0.028$, Wilcoxon signed rank test). This study demonstrated that myogenic activity significantly increases in the skin microcirculation during post local heating recovery compared to rest and local heating.

[PP22]

MAT.ANGIOPOIETIN-1 IMPROVES MICROCIRCULATORY PERFUSION DURING SEPSIS WITH AN IMMUNOMODULATORY EFFECT

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Sepsis is currently viewed as a complex dysregulation of inflammation due to an infection, leading to microcirculatory dysfunction; it has recently been reported that a depressed immunological surveillance greatly influences host survival and outcome [1]. This study investigated whether the Angiotensin-converting enzyme 2 (ACE2) variant MAT.Ang-1 [2] improved microcirculatory perfusion and local inflammation during sepsis. Aluminium chambers were implanted into the dorsal skinfold of male C3H/HeN mice to expose the striated muscle microcirculation. Ketamine/xylazine sedation was induced in (i) control, (ii) LPS (1 mg.kg⁻¹ ip. at 0 hrs and 19 hrs) and (iii) LPS + MAT.Ang-1 (33 µg iv. at 20 hrs) treated animals. At 24hrs microcirculatory blood flow was measured by laser Doppler flowmetry (MoorLDI2); the cytokine profile in abdominal muscle was determined by a multiplex array for simultaneous detection (R&D). LPS significantly reduced blood flow (Perfusion Units: 348.3±12.7 vs. 2036±542.6 in controls; p<0.05, n=5), being improved by the co-administration of MAT.Ang-1 (857.4±238.5, n=5). G-CSF and MCP-1 expression was reduced in striated muscle from septic mice and was restored by the MAT.Ang-1 injection. Therefore, MAT.Ang-1 may be beneficial in sepsis for maintaining tissue perfusion and stimulating host immune responses. (Funded by the BHF). [1] Spronk et al. (2004). *Critical Care* 8: 462-468; [2] Cho (2004). *Proc Nat Acad Sci* 101: 5547-5552.

[PP23]

BLOCKADE OF BOTH PLATELET GPIb AND P-SELECTIN IS ESSENTIAL TO ENSURE LONGER LASTING PROTECTION FROM INTESTINAL ISCHEMIA-REPERFUSION (IR) INJURY *IN VIVO*

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Introduction: We have demonstrated that blocking platelet GPIb prevents microthrombus formation which confers protection during early intestinal IR injury. However, microcirculatory and histological damage is present by 4 hrs post-reperfusion despite no thrombi being observed. It is possible that singular platelets and leukocytes contribute to this late onset of damage. Therefore, effects of blocking GPIb and/or P-sel on thrombus formation and individual platelet and leukocyte recruitment were monitored intravitaly for up to 4hrs post-rep. Methods: Anaesthetised (ketamine/xylazine) WT or P-sel^{-/-} mice underwent 45 mins ischemia. Labelled endogenous platelets (=thrombi), donor platelets (= individual platelets) and leukocytes were quantitated. Some platelets were pre-treated with an anti-GPIb antibody. Results: Inhibiting GPIb significantly ($p < 0.001$) prevented thrombus formation but led to a concomitant increase in leukocyte ($p < 0.001$) and individual platelet ($p < 0.05$) adhesion by 4 hrs post-rep. However, both these were significantly ($p < 0.05$) decreased in anti-GPIb ab+P-sel^{-/-} mice. This was associated with improved blood flow and tissue histology. Conclusion: Inhibiting both microthrombus formation and platelet-leukocyte-endothelial interactions is essential to ensure longer lasting improvement in gut microcirculation and histology. Therefore, therapeutic strategies targetting both GPIb and P-selectin may prove beneficial in improving the clinical morbidity associated with gut IR injury.

Supported by BHF

[PP24]

ADHESION OF PLATELETS DERIVED FROM MICE UNDERGOING INTESTINAL ISCHEMIA-REPERFUSION (IR) INJURY TO ENDOTHELIUM *IN VITRO*

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Introduction: We have previously demonstrated that microthrombi contribute to intestinal ischemia-reperfusion (IR) injury, with maximal platelet adhesion observed 10 min post-reperfusion. However, it is not known whether non-adherent platelets become activated whilst circulating through the injured gut, enabling their adhesion elsewhere. Therefore, adhesion of early (10 min IR) and late (120 min IR) reperfusion peripheral blood platelets to endothelium was monitored *in vitro* using flow-based adhesion assays. Methods: Platelets were isolated from anaesthetised (ketamine/xylazine) wild-type or P-selectin^{-/-} mice undergoing intestinal IR injury or sham surgery. Some platelets were activated with collagen prior to use. Platelets were flowed over stimulated (TNF α +TGF β) or naïve murine cardiac endothelial cells. Results: Collagen activated sham platelets significantly adhered to naïve (p<0.05) and activated (p<0.01) endothelium. 10 min IR platelets only adhered (p<0.01) to activated endothelium with adhesion being P-selectin dependent. 120 min IR platelets did not adhere to activated endothelium and adhesion could not be induced even with collagen activation. Conclusion: This study suggests platelets trafficking through inflamed gut can adhere in remote sites where endothelium may become activated by soluble cytokines. These events may contribute to remote tissue damage and subsequent multiple organ failure. Furthermore, for anti-platelet therapy to be effective, it must be introduced within a narrow time frame post-reperfusion.

[PP25]

TRANSFORMING GROWTH FACTOR- β 1 INDUCES MACROPHAGE MIGRATION INHIBITORY FACTOR EXPRESSION IN AORTIC SMOOTH MUSCLE CELLS

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Anti-inflammatory properties of transforming growth factor- β 1 (TGF- β 1) may account for its protective role against atherosclerotic plaque rupture. The transcription factor Nrf2 mediates induction of cytoprotective genes via activation of antioxidant response elements (ARE). Macrophage migration inhibitory factor (MIF) has multiple functions in inflammatory processes, exhibits catalytic thiol-protein oxidoreductase activity and plays a role in redox homeostasis. The present study investigates whether TGF- β 1 induces MIF expression in cultured wild-type (WT) and Nrf2 deficient (KO) mouse aortic smooth muscle cells (MASMC). In addition, nuclear translocation of Nrf2 and p53 were investigated in human aortic smooth muscle cells (HASMC) treated with TGF- β 1. Western blot analyses revealed that MIF expression were significantly enhanced by TGF- β 1 (0-10ng/ml, 0-12 h, $p < 0.01$, $n = 5$) in WT but not Nrf2 KO MASMC. TGF- β 1 (0-10 ng/ml, 2h) elicited significant ($p < 0.05$, $n = 3$) nuclear translocation of Nrf2 and attenuated nuclear accumulation of p53 in response to hydrogen peroxide generated by glucose oxidase (10 mU/ml, 2h) in HASMC. Our novel findings suggest that TGF- β 1 may afford protection in aortic SMC by enhancing Nrf2 mediated induction of anti-inflammatory protective genes.

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[PP26]

EFFECTS OF ESSENTIAL HYPERTENSION ON PLATELET FUNCTION, NITRIC OXIDE SYNTHASE AND ARGINASE IN PLATELETS

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Essential hypertension (EH) is a major independent cardiovascular risk factor. Nitric oxide (NO) is an important regulator of platelet function and nitric oxide synthase (NOS) and arginase pathways compete for the substrate, L-arginine [1]. We have previously reported an impairment of L-arginine influx and NO production in blood cells from EH patients [2]. The aim of this study was to investigate the effects of EH on L-arginine-NO pathway, urea cycle and function in human platelets. Arginase activity was analyzed through the conversion of [C¹⁴]-L-arginine into [C¹⁴]-urea. NOS activity was measured by the conversion of L-[³H]-arginine into L-[³H]-citrulline. The expression of inducible (iNOS) and endothelial NOS (eNOS) and arginase I and II were assayed by western blotting. Platelet aggregation induced by collagen and ADP was evaluated in platelet rich plasma by optical densitometry. Arginase I was detected in human platelets and its expression and activity were not significantly different between groups. NOS activity was diminished in EH but the expression of NOS isoforms was not affected. We also found an activation of platelet aggregability induced by collagen, but not by ADP. The thrombotic state observed in EH could be attributed to an impairment of NO bioavailability.

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[PP27]

MODERATE CHAMPAGNE CONSUMPTION PROMOTES AN ACUTE IMPROVEMENT IN ACUTE ENDOTHELIAL-INDEPENDENT VASCULAR FUNCTION IN HEALTHY HUMAN VOLUNTEERS

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Champagne wine has not been fully investigated for its cardioprotective potential. In order to assess whether acute and moderate Champagne wine consumption is capable of modulating vascular function in healthy human volunteers, we designed a randomized, crossover intervention trial. Fifteen volunteers consumed either Champagne wine or a control drink. Peripheral micro-vascular function was assessed using Laser Doppler imaging with iontophoresis. Consumption of Champagne wine was observed to induce an acute change in endothelium-independent vasodilatation, whilst the alcohol matched control did not induce any endothelium-independent changes. Although both Champagne wine and the control induced an increase in endothelium-dependent vascular reactivity, there was no significant difference between the vascular effects induced by Champagne wine or the control. These effects were accompanied by an acute decrease in the concentration of MMP-9, a significant decrease in the plasma levels of oxidising species and an increase in urinary excretion of a number of phenolic metabolites. Our data suggest that a moderate consumption of Champagne wine may improve vascular performance via the delivery of phenolic constituents capable of improving nitric oxide bioavailability and reducing MMP activity.

[PP28]

COMPARISON OF CUTANEOUS MICROVASCULATURE IN INDIAN ASIANS AND EUROPEANS: RELATIONSHIP TO DIABETES AND OTHER RISK FACTORS

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Background: People of Indian Asian descent have an increased risk of cardiovascular disease that cannot be explained by conventional risk factors. We investigated if microcirculatory function was impaired in an age, sex-matched population-based sample of people of Indian Asian descent compared with Europeans in UK. Methods: Cutaneous microvascular function was assessed using laser Doppler fluximetry in response to heating to 42°C (maximum hyperaemia) and 3 minute arterial occlusion (post occlusive reactive hyperaemia: PORH) in 148 Indian Asians and 147 Europeans. Blood pressure, anthropometry and fasting bloods were also measured. Results: Maximum hyperaemia and minimum resistance did not differ significantly by ethnicity. PORH was lower in Indian Asians and time to peak of PORH was prolonged. Diabetes was associated with reduced maximum hyperaemia and PORH. Adjustment for diabetes, accounted for differences in time to peak, but not differences in PORH (Europeans = 43.6 (39.7, 47.9); Indian Asians = 37.0 (33.6, 40.7); $p = 0.02$). Differences in risk factors did not account for interethnic differences in microvascular responses. Conclusion: Indian Asians have impaired microvascular function in response to ischaemia, that is partly, but not wholly explained by diabetes or other conventional risk factors. Abnormal microvascular function may contribute to elevated cardiovascular risk in Indian Asians.

[PP29]

HOW UBIQUITOUS IS NITRIC OXIDE VASOACTIVITY?

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The role of endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) for vasodilatation and/or angiogenesis in humans is equivocal. A lack of eNOS-specific inhibitor suitable for *in vivo* use means it is difficult to ascertain the importance of this NOS isoform *in vivo* in animals apart from mice, where gene ablation has shown that eNOS, but not neuronal (nNOS), is essential in the regulation of vascular tone, control of blood pressure [1] and angiogenesis [2]. However, whether this phenomenon exists in vertebrates other than mice is unclear. Indeed, there is no direct evidence that eNOS exists in some fish species including rainbow trout, common carp and zebrafish. Although non-specific NOS blockade with L-NNA inhibits angiogenesis in rainbow trout (unpublished) and reduces blood flow in zebrafish [3], whether the eNOS isoform is responsible has not been established. Further, isolated carp vessels do not dilate in the presence of sodium nitroprusside (SNP) or exogenous cyclic guanine monophosphate (cGMP), suggesting that the vascular NO-cGMP pathway does not exist in this species. In addition, there are no sequences in the literature for an eNOS gene in any fish species, and PCR performed on three fish species produces differing products. However, blood pressure in fishes is lower than in mammals, suggesting potent vasodilator activity exists. These observations suggest that the role of eNOS in blood flow and angiogenesis may not be the same in all vertebrates.

[1] Shesely *et al* (1996) *PNAS* 93:13176

[2] Williams *et al* (2006) *J Physiol* 570:445

[3] Fritsche *et al* (2000) *Am J Physiol* R279:2200

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ENDOTHELIUM AND VASCULAR CELL FUNCTION / DYSFUNCTION

[PP30]

EFFECT OF PERIPHERAL VASCULAR DISEASE ON ENDOTHELIAL FUNCTION IN HUMAN SKELETAL MUSCLE RESISTANCE ARTERIES

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Peripheral arterial disease (PAD) is associated with altered blood flow dynamics and altered microvascular function. In this study mechanisms of endothelium-dependent and endothelium-independent function in human skeletal muscle resistance arteries were undertaken to identify potential mechanism of microvascular dysfunction contributing to ischaemia. Resistance arteries were isolated from 18 male subjects with PAD from non-ischaemic and ischaemic skeletal sites (Lumen dia: $169 \mu\text{m}$ vs. $177 \mu\text{m}$ respectively). Structural analysis using confocal microscopy confirmed hypotrophic remodeling in ischaemic arteries vs. non-ischaemic. Despite chronic ischaemia intact endothelium was observed with no difference in ischaemic vs. non-ischaemic. Vasorelaxation measures were undertaken using pressure myography. PAD produces significant endothelium-dependent dysfunction. Relative to non-ischaemic endothelial dysfunction appears exclusive to endothelium-derived nitric oxide. Endothelium-derived hyperpolarizing factor appears unaffected by PAD. The endothelium-dependent dysfunction can be correlated with loss of total endothelial nitric oxide synthase (eNOS), reduced phosphorylation of eNOS and reduced ability to generate cGMP. These data provide a first time insight into the mechanism underlying significant loss of vascular homeostatic control mechanisms in human skeletal muscle resistance arteries. This work adds to our understanding of vascular pathogenesis in PAD highlighting significant involvement of resistance arteries as a secondary consequence of the large artery disease.

[PP31]

INTERACTION OF NITRIC OXIDE SYNTHASE TYPE 3 WITH BETA-CATENIN IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Nitric oxide synthase type 3 (NOS-3) can undergo activation, classically via a Ca^{2+} -calmodulin dependent pathway or via Ca^{2+} -independent mechanisms involving phosphorylation of key residues and association with a number of regulatory proteins. The aim of the present study was to determine whether the integrin beta-catenin associates with NOS-3 in endothelial cells, and whether such association gives rise to nuclear translocation of beta-catenin, since such translocation of beta-catenin can have important effects on transcription of certain genes. Human umbilical vein endothelial cells (HUVEC) from normal pregnancies were treated with histamine (100 μM), thrombin (1U/ml), salbutamol (1 μM), adenosine (100 μM) or vehicle, for 2 min at 37°C. Immunoprecipitation (IP) was employed to detect association of NOS-3 and beta-catenin. IP of beta-catenin resulted in co-precipitation of NOS-3, and similarly NOS-3 IP resulted in co-precipitation of beta-catenin, confirming a co-association of these two proteins. Moreover, their co-association was increased by histamine (208 \pm 114%), salbutamol (222 \pm 110%), adenosine (207 \pm 113%) and thrombin (211 \pm 112%) ($p < 0.01$ for each, $n = 6$). In addition, immunoblotting of nuclear extracts confirmed an increase in nuclear beta-catenin in response to the NOS activators tested. These data demonstrate that beta-catenin associates with NOS-3, and NOS-3 activation may cause both increased co-association and nuclear translocation of beta-catenin; the functional consequences of this remain to be determined.

[PP32]

ROLE OF MICRO RNA-503 IN DIABETIC ENDOTHELIOPATHY

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Diabetes disturbs endothelial function and reparative neovascularisation, thus contributing to limb ischemia. Recently, we showed that the atypical neurotrophin receptor p75^{NTR} mediates diabetes-determined impairment of reparative neovascularization. Diabetes induces p75^{NTR} expression in microvascular endothelial cells (ECs) of ischemic limb muscles, thus impairing EC survival and functions. Making a step forward into the understanding of the relevance of p75^{NTR} in diabetic microangiopathy, here we studied whether p75^{NTR} modulates the expression of microRNAs (miRNAs) in ECs. Using microarray analyses of miRNAs expression, we detected that miRNA-503 (miR-503) was consistently induced in p75^{NTR}-transduced human umbilical vein ECs (HUVECs). Moreover, both p75^{NTR} and miR-503 expression in HUVECs was increased following culture in high glucose to mimic diabetic hyperglycaemia. Furthermore, miR-503 expression was higher in ischemic muscles of diabetic mice in comparisons to non diabetic mice. Overexpression of miR-503 in HUVECs impaired cell proliferation and decreased *in vitro* capillary-like network formation on matrigel. Using *in silico* and *in vitro* analyses, we identified CyclinE1, cdc25A and VEGF-A as direct targets of miR-503 in ECs. Moreover, inhibition of miR-503 restored proper post-ischaemic neovascularization in diabetic mice. These results suggest a role of miRNA-503 in diabetes-induced microangiopathy and impaired angiogenesis.

[PP33]

INFECTION OF ENDOTHELIAL CELLS WITH KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS SELECTIVELY INHIBITS NEUTROPHIL RECRUITMENT IN AN INFLAMMATORY MODEL

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Kaposi's sarcoma (KS) is an endothelial cell (EC) tumour, caused by Kaposi's sarcoma-associated herpesvirus (KSHV). KS has a pronounced, predominantly lymphocytic, inflammatory component. KSHV manipulates EC expression of adhesion receptors, cytokines and chemokines, such as ICAM-1, VCAM-1 and IL-8. Therefore, we hypothesized that KSHV infection may induce or modulate an inflammatory response in EC, and thus influence leukocyte recruitment. To test this hypothesis, human umbilical vein EC were infected with KSHV and cultured for between 4h and 10 days, prior to stimulation with tumour necrosis factor- α (TNF) or interleukin-1 (IL-1). Neutrophils or peripheral blood lymphocytes (PBL) were then perfused over the EC, and their adhesion and migration were observed by phase-contrast video-microscopy. KSHV-infection alone did not induce leukocyte recruitment, or alter the number of neutrophils or PBL binding from flow to stimulated EC. However, infection significantly decreased neutrophil transendothelial migration, particularly in response to TNF (~75% inhibition), an effect seen from 24h post-infection onwards. Transendothelial migration of PBL was unaffected. This is the first description of a physiologically-significant functional change to KSHV-infected EC, which may provide a viral survival advantage and contribute to KS pathogenesis.

[PP34]

LOSS OF ISLET ENDOTHELIUM IN VITRO IS INDEPENDENT OF VEGF-A SPLICE VARIANT EXPRESSION

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Heterotypic interactions between endocrine cells and vascular endothelial cells in islets are important for regulating islet development and function. Identification of the molecular mechanisms maintaining intra-islet cell:cell communication may inform strategies for enhancing islet revascularisation and improving the outcome of islet transplantation. We have investigated the profile of angiogenic molecules expressed by human and mouse islets in vitro. Following isolation islets displayed a rapid reduction in the mRNA and protein expression of several endothelial cell (EC) markers, including CD105 and Vascular Endothelial Growth Factor (VEGF) receptor 2. Islet-conditioned media (containing 2mM or 20mM glucose) reduced EC viability and modulated EC gene expression. The rapid loss of EC markers occurred despite maintained expression of cytoprotective isoforms of VEGF-A (120/121, 164/165 and 188/189) which were all expressed at high levels throughout a 7 day culture period. Similarly, islet HIF-1 alpha expression was sustained during culture and a pharmacological agent which promotes HIF-1 alpha degradation did not prevent EC loss, suggesting that loss of islet ECs during culture is also independent of HIF-1 alpha. Further understanding of the mechanisms involved in the loss of EC populations will enable the development of therapeutic strategies for ensuring the rapid engraftment and survival of the islets in the post-transplantation period.

[PP35]

HEPARAN SULFATE COMPONENT OF ENDOTHELIAL GLYCOCALYX CONTRIBUTES TO THE WATER PERMEABILITY OF MESENTERIC MICROVESSELS

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Endothelial glycocalyx A) comprises lumenally-bound glycoproteins, proteoglycans, glycosaminoglycans (GAGs), and plasma components, B) lines all blood vessels and C) regulates permeability [1]. Heparinase-III (Hep3) cleaves hexosamine from glucuronic acid residues in GAGs [2], thereby removing heparan sulfate (HS). We sought to examine the contribution of glycocalyx-bound HS to permeability *in vivo*.

L_p (hydraulic conductivity, or water permeability) of mesenteric microvessels of MS222-anesthetized *Rana pipiens* was measured with the Landis-Michel technique. Bovine serum albumin (BSA) perfusion (baseline) was followed by 15min perfusion with either $0.1 \text{ U} \cdot \text{mL}^{-1}$ Hep3 or BSA alone (control). L_p was determined by measuring fluid flux at known luminal hydrostatic pressures before and after Hep3 or control perfusion. L_p ($\times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{cmH}_2\text{O}^{-1}$; mean \pm s.e.m.(n)) rose following Hep3 perfusion (baseline: 4.3 ± 1.1 (9); Hep3: 17.0 ± 7.0 (9)) but was unaltered by control perfusion (baseline: 4.4 ± 0.7 (8); BSA alone: 4.4 ± 0.7 (8)). Perfusion with Hep3 therefore causes a significant 3.8 ± 0.9 fold increase in L_p ($p < 0.05$ vs control perfusion, Mann-Whitney). Heparan sulfate within the endothelial glycocalyx is an important determinant of microvascular permeability *in vivo*: changes in the endothelial glycocalyx in disease may therefore result in altered permeability.

[1] Michel & Curry, *Physiol Rev* 1999 [2] Nader, *Biol Chem* 1990

RETINAL MICROCIRCULATION / OPHTHALMOLOGY

[PP36]

RETINAL ENDOTHELIAL CELL GLYCOCALYX CHANGES IN HIGH GLUCOSE – ROLE OF HEPARANASE 1

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Background: Diabetic retinopathy (DR) remains a leading cause of blindness in working-age adults. Growing evidence suggests that DR is an inflammatory condition associated with increased entrapment of leukocytes in the retinal microcirculation. This process of leukostasis leads to limited blood flow and the development of DR. There is evidence that the glycocalyx on the endothelial surface influences the leukocyte-endothelial cell adhesion. Glycocalyx heparan sulfate proteoglycans can be degraded and the glycocalyx modified by heparanase 1 (HPSE) which is involved in inflammation, angiogenesis, vascular permeability, and wound healing. Aim: This study aimed to investigate whether high glucose affects the structure of retinal capillary endothelial cell glycocalyx via HPSE release. Methods: Bovine retinal endothelial cells (BREC) were exposed to normal (NG, 5.6mM), high glucose (HG, 25mM) or mannitol (25mM) for 24h and 4 days, and the glycocalyx labelled with FITC-conjugated wheat germ agglutinin and assessed by fluorescence microscopy and a cell based fluorescence assay. Intracellular and extracellular levels of HPSE were determined by Western blotting. Results: Glycocalyx was reduced [84±9.6% vs. NG (100%), $p < 0.001$, $n = 15$] after acute (24h) exposure to HG. HG and mannitol significantly increased HPSE secretion to 185±89% ($p < 0.001$, $n=17$) and 139±72 ($p=0.0048$, $n=17$) vs. NG (100%), respectively. Conclusion: Altered glycocalyx structure and function via glucose-induced HPSE expression may play a role in leukostasis and the pathogenesis of early DR.

[PP37]

THE RECEPTOR FOR AGES (RAGE) AND COGNATE LIGANDS IN SUBRETINAL LESION DEVELOPMENT IN A MURINE MODEL OF OCULAR NEOVASCULARISATION

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RAGE is a well-characterized pattern-recognition receptor that evokes pro-inflammatory cytokine expression, and can be linked to choroidal neovascularisation (CNV). We have investigated a definitive role for RAGE by control of microglial infiltration and angiogenesis in the subretinal space in a murine model of CNV pathology. C57Bl/6 wild-type (WT) and RAGE knockout mice (RAGE^{-/-}) (n=8/group) were subjected to diode laser photocoagulation (3 burns, 50µm spot size), rupturing Bruch's membrane. Retinal flatmounts were analysed for CNV and several infiltrating microglia expression markers. Animals were anaesthetised with a combination of 1% ketamine/xylazine. As previously reported, CNV lesion size reached maximal diameter at 2 weeks in WT mice, with RAGE^{-/-} mice exhibiting reduced CNV lesion size compared to control (p<0.05) and lesion regression by 10 days on average. Three different microglia populations were identified within and proximal to lesions. Active microglia were higher in number in CNV mice compared to non-CNV controls. RAGE^{-/-} mice showed significantly less microglia (active phenotype) compared to WT control. S100B-immunoreactivity was high in CNV lesions and S100B caused cytokine release from microglia and evoked phosphorylation of the p44/42 pathway, a response blocked by neutralising antibody. This study illustrates a key function of RAGE signalling in CNV development and RAGE blockade could represent an important therapeutic strategy.

[PP38]

RETINAL VASCULAR PLEXUS REMODELLING OCCURS BY A CO-ORDINATED PROCESS OF ENDOTHELIAL CELL APOPTOSIS

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Introduction: In the first three post-natal weeks, the murine retinal vascular plexus (RVP) forms as endothelial cells migrate from the optic nerve head (ONH) across the retinal surface towards the periphery. The initially dense plexus is remodelled by a progressive outward radial wave of endothelial cell apoptosis [1]. We hypothesise an inverse relationship between the distance of Caspase-3 positive endothelial cells from the ONH and their distance from the nearest arteriole: implicating vessel flow (and therefore localised oxygen tensions) in the remodelling process. Methods: Mice were euthanized by isoflurane overdose and cervical resection. Using whole-mount immunofluorescence for endothelial cells (GSI-B4) and Caspase-3 the distance of each dying endothelial cell from the ONH and from the nearest arteriole was measured to determine the spatio-temporal patterning of cell death. Results: Caspase-3 positivity can be seen in all endothelial cells of individual segments at the edge of the remodelling plexus. No significant correlation was found between their distances from the ONH and from the nearest retinal arteriole. Discussion: The patterning of endothelial cell Caspase-3 positivity suggests that blood vessel segments in their entirety are targeted for removal during RVP remodelling. Quantitative data relating endothelial cell death to both flow and pericyte coverage will be presented.

[1] Gerhardt *et al.* (2003). *J Cell Biol* **161**: 1163-1177

[PP39]

NADPH OXIDASE DERIVED ROS CONTRIBUTE TO THE PATHOGENESIS OF DIABETIC RETINOPATHY

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Although, oxidative stress plays a role in the pathogenesis of diabetic retinopathy (DR) the source and location of glucose-induced reactive oxygen species (ROS) is unclear. Studies using macrovascular endothelial cells showed that mitochondrial ROS linked hyperglycaemia and biochemical pathways responsible for glucose-induced vascular damage. Using apocynin, a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor, and MitoQ, a mitochondrial antioxidant, we addressed the importance of mitochondria vs. NADPH oxidase-derived ROS in glucose-induced apoptosis of bovine retinal pericytes (BRP). Immunoblot analysis and cytochrome C assay identified the presence of active NADPH oxidase in BRP. Immunogold cytochemistry showed that NADPH oxidase is present in the cytoplasm, suggesting that ROS is produced intracellularly in BRP. High glucose (25mM) significantly increased the number of apoptotic cells by 122% of normal glucose (5mM), as evaluated by caspase-3 activity. Exposure to high glucose significantly increased intracellular glucose concentration and N^ε-(carboxymethyl) lysine (CML) content, a marker of oxidative stress. Apoptosis was associated with increased gp91phox expression, NADPH oxidase activity and intracellular ROS production. Treatment with apocynin but not MitoQ significantly blunted the generation of ROS, formation of CML and induction of caspase-dependent apoptosis induced by high glucose. These results suggest that NADPH oxidase and not mitochondria-derived ROS is responsible for the accelerated apoptosis of pericytes in DR.

[PP40]

NADPH OXIDASE SIGNALLING IN TNF- α -INDUCED ACTIVITY OF CORE 2 β -1, 6-N ACETYL GLUCOSAMINEYLTRANSFERASE IN DIABETIC LEUKOCYTES

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Background: Diabetic retinopathy (DR) is an inflammatory condition with increased leukostasis leading to capillary non-perfusion. Glycosylation on the surface of leukocytes by the enzyme core 2 β -1, 6-N-acetylglucosamineyltransferase (C2GNT) influences leukocyte-endothelial cell adhesion. Aim: Investigate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase signalling in the tumour necrosis factor- α (TNF- α)-induced activity of C2GNT in diabetic leukocytes. Methods: Human leukocytes (U937) and a p47phox-deficient lymphoblastoid cell line (FI0007) were exposed to TNF- α (8pg/ml) for 24h in the presence and absence of (1) NADPH oxidase inhibitors, (2) PKC β 1/2 inhibitor or (3) antioxidants. C2GNT and NADPH oxidase activities were measured. Results: Compared to control media TNF- α treatment raised C2GNT [927.5 \pm 199.4 vs.166.5 \pm 54.16 (n=12), P<0.001] and NADPH oxidase [100 \pm 0 vs.142.0 \pm 16.89 (n=5), P<0.005] activity in U937 cells, which was significantly reversed by treatment with antioxidants, PKC β 1/2 and NADPH oxidase inhibitors. TNF- α treatment of FI0007 cells failed to increase C2GNT and NADPH oxidase activity. Transfection of FI0007 cells with p47phox cDNA resulted in the C2GNT and NADPH oxidase response to TNF- α being restored [400.1 \pm 164.4 vs.990.4 \pm 294.9 (n=10), P<0.02]. Conclusion: These results demonstrate a novel signalling crosstalk between TNF- α , GCNT1, NADPH oxidase and PKC β 1/2 in diabetic leukocytes.

[PP41]

THE ACTIVITY OF THE INFLAMMATORY ENZYME C2GNT [β 1,6 N-ACETYLGLUCOSAMINYLTRANSFERASE ARE RAISED IN PATIENTS WITH DIABETIC NEPHROPATHY

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Objective: Diabetic nephropathy (DN) is the main cause of end-stage renal diseases, but the exact mechanisms leading to the development and progression of renal injury are not yet fully understood. Since there is evidence suggesting that chronic, sub-clinical inflammation plays a role in the pathogenesis of diabetic nephropathy we examined if the inflammatory enzyme, core 2 β -1, 6-N-acetylglucosaminyltransferase (C2GNT) is associated with DN. We have already established the role of C2GNT in diabetic retinopathy and neuropathy. Methods: This randomised and controlled investigation included diabetic patients without nephropathy (n=10), diabetic patients with nephropathy (n=8) and age-matched healthy control subjects (n = 12). Whole blood was drawn and collected in ethylenediaminetetraacetic acid tubes. Polymorphonuclear (PMN) leukocytes and plasma were isolated using density gradient centrifugation. The PMNs were lysed and the C2GNT activity assessed. Results and Conclusion: The activity of C2GNT was significantly higher in PMNs of patients with diabetes as compared to age-matched healthy control subjects [267.4 ± 139.3 vs. 22.10 ± 15.05 pmoles/h/mg protein, $P < 0.05$]. Moreover, the C2GNT activity was significantly higher in patients with nephropathy compared to age matched healthy controls [1973 ± 1475 vs. 267.4 ± 139.3 pmoles/h/mg protein, $P < 0.05$]. Higher activity of C2GNT in leukocytes supports the important role of inflammation in diabetic nephropathy.

[PP42]

SPLICING FACTOR POLYMORPHISMS, THE CONTROL OF VEGF ISOFORMS AND ASSOCIATION WITH ANGIOGENIC EYE DISEASE

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Vascular Endothelial Growth Factor (VEGF) is a key element in the balance of pro- and anti-angiogenic growth factors in Proliferative Diabetic Retinopathy (PDR) and has been suggested in exudative Age-related Macular Degeneration (exAMD). Three splicing factors, SRp40, SRp55 and ASF/SF2 are predicted to control alternative splicing of VEGF by binding to exonic splice enhancers (ESE) in the *VEGF* gene. In this study we assessed potential associations between angiogenic eye disease and splicing factor polymorphisms and screened for sequence variations in the alternatively spliced region of the *VEGF* gene. This was a case:control study comparing 69 PDR and 94 exAMD patients with 95 age-matched controls. Splicing Factor polymorphisms were genotyped by RFLP and sequencing, and the VEGF alternatively spliced region was assessed by dHPLC. ASF/SF2 polymorphisms showed no association with exAMD or PDR. We observed a significant association in SRp55, 2994 C>T (rs2235611), where the C allele was more common in the PDR group ($p=0.03$). We also observed a trend in SRp40 5136 a>c (rs6573908) where the CC genotype was more frequent in controls ($p=0.0517$). No variations were observed in VEGF alternatively spliced region. The link between PDR and the SRp55 2994 polymorphism may suggest a disease specific association between splicing factor known to control VEGF splicing and proliferative diabetic retinopathy.

NEPHROPATHY

[PP43]

LIPOPOLYSACCHARIDE INDUCTION OF ALDOSE REDUCTASE IN PATIENTS WITH TYPE 1 DIABETES AND DIABETIC NEPHROPATHY

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Aim: There is growing evidence that diabetic nephropathy (DN) is an inflammatory disease. Aldose reductase (AKR1B1) is known to mediate cytotoxic signals induced by high glucose (HG); it has also been suggested to mediate inflammatory signals. Aim of this study was to look at the effects of inflammation on the expression levels of AKR1B1 in patients with type 1 diabetes (T1D) with and without DN. This was achieved by exposing the cells from the patients to lipopolysaccharide (LPS), a potent pro-inflammatory mediator and HG. **Methods:** Peripheral Blood Mononuclear Cells (PBMCs) were extracted from 20 Caucasoid patients with T1D; 10 uncomplicated and 10 nephropaths. PBMCs were cultured for three days in either normal (NG) (5.5mM) or high glucose (HG) (25mM), LPS was added for the final 24 hours (10 μ g/m). AKR1B1 mRNA and protein levels were measured using Ribonuclease Protection Assay and Western Blotting respectively. **Results:** There was a significant increase in AKR1B1 mRNA (1.54vs.3.27, $p<0.001$) and protein (4.25vs.7.02, $p<0.005$) in response to HG in the nephropaths. There was no response in the uncomplicated. A similar pattern in AKR1B1 mRNA and protein expression was seen in response to LPS under both NG and HG; nephropaths (NG: 1.54vs.2.46, $p=ns$; 4.25vs.6.8, $p<0.01$ respectively; HG: 3.27vs.4.99, $p<0.001$; 7.02vs.10.91, $p<0.01$ respectively). There was no response in the uncomplicated. **Conclusions:** Increase in AKR1B1 in response to HG and LPS in patients with T1D and DN may result in their cells being exposed to a combination of increased reactive oxygen species and inflammatory mediators leading to cellular and tissue damage.

[PP44]

ACTIVATION OF AMPK – IMPLICATIONS FOR DIABETIC NEPHROPATHY

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Renal Hypertrophy is the earliest sign of renal complications in diabetes, the mammalian target of Rapamycin (mTOR) pathway which feeds into mRNA translation may be a target for reducing early renal hypertrophy. 5' adenosine monophosphate-activated protein kinase (AMPK), part of the mTOR pathway, coordinates input from energy stressors and from the drug Metformin. Activation of AMPK inhibits mTOR leading to a reduction in the translation of extracellular matrix mRNA. The aim of this study was to investigate the effect of glucose concentration and treatment with Metformin on AMPK activation in a renal cell model. HEK293 cells were cultured in normal (5.5mM) and high (25mM) glucose concentrations and treated with Metformin at a range of concentrations (0-20mM). Activation of AMPK and mTOR was assessed using western blotting. Antibodies against phospho AMPK (T172), total AMPK, phospho mTOR (S2448) and mTOR were used as markers of AMPK and mTOR activation. Treatment of HEK293 cells with 20mM Metformin under conditions of low glucose (5.5mM) results in a 14 fold increase in AMPK activation. This is coupled with a decrease in active mTOR to 40% of the baseline value. Under high glucose (25mM) increasing concentrations of Metformin (0-20mM) seem to have little effect on AMPK activation. This suggests that although Metformin activates AMPK under low glucose this is inhibited by higher levels of glucose. Preliminary data suggest that Metformin may be useful in inhibiting renal hypertrophy caused by increased extracellular matrix deposition by reducing mRNA translation.

[PP45]

ATORVASTATIN PLUS AMLODIPINE/PERINDOPRIL DELAYS DECLINE IN e-GFR IN DIABETES. A SUBSTUDY OF THE ANGLO-SCANDINAVIAN CARDIAC OUTCOMES TRIAL(ASCOT)

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Objective: The role of statins in diabetic renal microvascular disease is unclear. We determined the effects of blood pressure lowering and statin on estimated glomerular filtration rate (eGFR) in people with and without diabetes. *Methods:* Randomised controlled trial of 2 antihypertensive regimes (amlodipine±perindopril vs atenolol±bendroflumethiazide). Those with total cholesterol≤6.5mmol/l were further randomised to receive atorvastatin or placebo(2x2 factorial design). We studied 600 men and women participating in the intensively phenotyped substudy of ASCOT. Participants were aged 40-79 years with hypertension plus at least 3 additional cardiovascular risk factors. e-GFR was calculated at baseline and after 3.3 yrs. *Results:* Overall, eGFR declined from 70.2±11.8 to 67.7±12.4 ml/min/1.73² (p <0.001). However, those receiving amlodipine/perindopril+atorvastatin were protected from the decline observed in all 3 other treatment groups, (final eGFR adjusted for baseline eGFR=70[95% CI:69,71], interaction p=0.025).The protective effect of amlodipine/perindopril+atorvastatin was most apparent in those with diabetes (n=100,interaction p=0.03) *Conclusion:* Concurrent treatment with amlodipine/perindopril + atorvastatin delays decline in eGFR over 3 years of follow-up in hypertensive individuals without hypercholesterolaemia. This effect was most marked in people with diabetes.

ANGIOGENESIS – BASIC AND CLINICAL ASPECTS

[PP46]

IMPROVEMENT OF STOMACH PERFUSION FOLLOWING ISCHAEMIC CONDITIONING IN PREPARATION FOR MINIMALLY INVASIVE OESOPHAGECTOMY

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Background: Minimally invasive oesophagectomy (MIO) is a valid, less traumatic alternative to open surgery for the management of oesophageal cancer. However, a significant incidence of ischaemia-related gastric conduit failure (GCF) is observed with this approach. Laparoscopic Ischaemic conditioning (LIC) of the stomach, via neo-vascularisation, is believed to have a protective role, so we explored this potential benefit. Methods: MIO has been our standard procedure since April 2004. Comprehensive surgical data is collected prospectively and this was analysed. LIC was offered to patients on "informed consent basis". This involved a ligation of the left gastric artery at staging laparoscopy, 2 weeks prior to MIO. GCF is of 3 Types: TI (simple leak managed conservatively); TII (tip necrosis) and TIII (total ischaemia) require surgical intervention. Results: As of June 2009, 118 patients underwent an MIO. Inpatient mortality was 3.4% (4/118). 16.1% (19/119) patients developed GCF (9TI, 5 TII, 5 TIII). 59 patients did not undergo LIC and 20.3% (12/59, 4TI, 5TII, 3TIII) developed GCF. Of the 59 patients who underwent LIC, only 11.9% (7/59, 5TI, 2TIII) developed GCF. There was no incidence of TII failure after LIC, $p=0.022$. Conclusions: In this clinical setting, Laparoscopic Gastric Ischaemic Conditioning appears to protect against GCF, with elimination of TII failure. Further validation of this benefit is mandated and we have started a randomised controlled trial (LOGIC) to assess change in perfusion of the stomach using laser Doppler fluximetry.

[PP47]

IL-4, IL-13 OR PGE2 CONDITIONED M2 MACROPHAGES EXPRESS DIVERGENT ANGIOGENIC PROPERTIES INDICATED BY sFLT-1/ VEGF RATIO

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Macrophage activation by IL-4 and IL-13 generates an arginase expressing M2 phenotype, which is observed during wound healing and parasitic infection. Prostaglandin E2 (PGE2) mediated M2 conditioning strongly induces vascular endothelial growth factor (VEGF) from macrophages. However, the contribution of anti-angiogenic macrophage mediated soluble VEGF receptor 1 (sFlt-1) level remains poorly understood. To interrogate this, bone marrow derived macrophages (BMDMs) were treated by PGE2 with/without IL-4 or IL-13. VEGF and sFlt-1 protein and mRNA expression were determined. HUVEC proliferation assays were performed to validate sFlt-1 production. Arginase-1 mRNA was increased in IL-4, IL-13 or PGE2 treatment. Soluble Flt-1 levels were markedly upregulated following IL-4 or IL-13 conditioning. Such conditions inhibited PGE2-induced VEGF production even though the extent of sFlt-1 expression was less than when BMDM were conditioned in IL-4 or IL-13 alongside PGE2, and corroborated by reduced HUVEC proliferation. This data shows that Th2 cytokines (IL-4 and IL-13) stimulate a macrophage sFlt-1^{hi} and arginase-1^{hi} M2 phenotype. In contrast PGE2 alone generates a VEGF^{hi} and sFlt-1^{lo} signature. High sFlt-1/ VEGF ratios indicate that IL-4 or IL-13 conditioned macrophages have anti-angiogenic properties, independent of M2 arginase-1⁺ phenotype and able to counter the angiogenic drive of PGE2-induced M2 macrophage-mediated VEGF production.

[PP48]

THE RECEPTOR FOR AGES (RAGE) REGULATES MICROGLIAL INFILTRATION AND LESION DEVELOPMENT IN A MURINE MODEL OF CHOROIDAL NEOVASCULARISATION (CNV)

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Activation of pro-inflammatory pathology is linked to wet age-related macular degeneration (AMD) and CNV. This study has sought to establish if RAGE has a role in CNV by controlling microglia infiltration and associated angiogenesis in the sub-retinal space. C57Bl/6 wild-type (WT) and RAGE knockout mice (RAGE^{-/-}) (n=8/group) were anaesthetized and subjected to diode laser photocoagulation (50µm spot size, 0.10s duration, 250 mW) to produce CNV. Retinal flatmounts were analysed for CNV and microglia. In a parallel in vitro study, microglia were exposed to the RAGE ligand S100B and signal transduction leading to cytokine expression assessed. CNV lesion size is maximum at 2 weeks in WT while RAGE^{-/-} exhibited significantly reduced size at the same time point (p<0.05). Three different populations of microglia (ramified/ inactive, divergent/activating and amoeboid/active) were identified within and proximal to CNV lesions. There were also significant differences in the number of different microglia between WT and RAGE^{-/-}. S100B immunoreactivity was high in CNV lesions and microglia exposed to protein in vitro upregulated RAGE protein. S100B evoked phosphorylation of the Erk1/2 pathway and also caused cytokine release from microglia. This study demonstrates a key role for RAGE in CNV by regulation of infiltrating microglia and pro-inflammatory signalling and blockade of RAGE could be a therapeutic option for retinal inflammation.

[PP49]

ANGIOPOIETIN-INDUCED NEUTROPHIL MIGRATION *IN VITRO* AND *IN VIVO*

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The angiopoietin family of growth factors ligate to the endothelial tyrosine kinase receptor Tie2. Ang-1 is agonistic for Tie2 and plays a key role in blood vessel maturation and stability as well as demonstrating anti-inflammatory properties. Ang-2 is classically thought of as an antagonistic ligand which counteracts the effect of Ang-1. Recently, Tie2 expression was demonstrated on human neutrophils, allowing these cells to respond to the angiopoietins. We hypothesise that the angiopoietins signal through Tie2 on neutrophils to modulate migration. The aims were to measure the effects of both angiopoietins on isolated mouse neutrophil migration *in vitro* and *in vivo*. Tie2 expression was confirmed on isolated mouse neutrophils by immunohistochemistry, flow cytometry and Western blot. Isolated neutrophils migrated to each of the angiopoietins and this was significantly ($p < 0.01$) decreased after treatment with a Tie2 blocking antibody. Blocking antibodies directed against CD18 and L-selectin demonstrated significant ($p < 0.001$ and $p < 0.1$ respectively) inhibition of angiopoietin-induced neutrophil migration. *In vivo* experiments also showed mouse neutrophil migration in response to intraperitoneal Ang-1 variant MAT-Ang-1 (33 $\mu\text{g}/\text{mouse}$) (gifted by Dr Richard Kammerer, University of Manchester). The development of a Tie2 knockdown mouse model, using lentivirus shRNA-mediated silencing of bone marrow expressed-Tie2, will be used to further investigate the effects of the ang/Tie2 system on the early stages of the inflammatory process in a physiologically relevant context.

[PP50]

CLASS 3 SEMAPHORINS AND THEIR PLEXIN RECEPTORS IN HUMAN DERMAL WOUND HEALING

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Angiogenesis in wound healing is a complex process, during which guidance cues for activated endothelial cells are regulated by chemorepulsive signals. Class 3 semaphorins (Sema3) bind to neuropilin 1, 2 and plexins and these interactions are known to induce repulsion in neurons and are hypothesised to inhibit angiogenesis causing vascular regression, but the role of Sema3 and plexins have yet to be studied. Therefore we characterised blood vessel formation and the spatial and temporal expression of plexins and their ligands, Sema3 in scar biopsies obtained from patients between 3 days and 2 years post surgery (n=96) and control skin (n=21). Histological analysis revealed that CD34 (newly formed blood vessels), CD105 (proliferating blood vessels) and CD133 (endothelial progenitor marker) expression were all significantly increased in early scars compared to control skin ($P<0.05$) and significantly related to scar age ($p<0.05$). In contrast there was a decrease in vessel α -SMA expression. Sema3A expression was not observed in blood vessels and only a weak to moderate expression of Sema3F, Plexin A1 and Plexin D1 was observed in all tissues. In contrast the expression of Plexin A3 and Sema3B was significantly increased in scar tissues during the vascular regression stage ($p<0.05$). These data indicate an increase in angiogenesis between 2 and 24 weeks following surgery and a vascular regression phase from 25 weeks onwards. Sema3B and PlexinA3 may be involved with vascular regression.

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[PP51]

CLASS 3 SEMAPHORINS INHIBIT ANGIOGENIC ACTIVITY *IN VITRO*

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Class 3 semaphorins are neuronal guidance molecules, which induce repulsion and growth cone collapse in neurons via binding to neuropilins 1 & 2 and plexin receptors. Two pro-angiogenic molecules, VEGF and PDGF-BB, also bind to neuropilins, therefore we hypothesise that class 3 semaphorins may regulate angiogenesis by preventing binding of VEGF and/or PDGF-BB to neuropilins. This study therefore assessed the effects of recombinant Class 3 semaphorins (Sema3A and 3F) on human dermal microvascular endothelial cell (HuDMEC) proliferation (MTS assay), migration (scratch assay) and tubule formation (Matrigel assay) *in vitro* in the presence and absence of VEGF-A and PDGF-BB. Neither semaphorin 3A or 3F had a significant inhibitory effect on HUDMEC proliferation. In contrast both class 3 semaphorins significantly ($P < 0.05$) inhibited HuDMEC migration in a dose dependent manner, and significantly decreased the number of tubules, proliferating islands and branch points in the Matrigel tubule formation assay ($P < 0.05$) in the presence and absence of VEGF or PDGF-BB. These results suggest that semaphorin 3A and 3F may inhibit the angiogenic activity of VEGF and PDGF by inhibiting endothelial cell migration and tube formation. Work is currently underway to establish whether this is due to preventing VEGF and PDGF from binding to neuropilins.

This work is funded by the Sheffield Teaching Hospitals Charitable Trust

[PP52]

THE POTENTIAL ROLE OF PLATELETS IN ENDOTHELIAL PROGENITOR CELL ADHESION DURING ANGIOGENESIS

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Endothelial progenitor cells (EPCs) are implicated in the angiogenic response to vascular injury as well as the continual maintenance of endothelium integrity [1]. It is suggested that adherent platelets have a pivotal role in EPC recruitment at sites of vascular repair. Platelets express surface ligands for a range of adhesion receptors and secrete potent chemokines. These are potential mediators of EPC homing to endothelium through formation of platelet-EPC bridges [2]. We investigated EPC-platelet binding using cell-based aggregometry and a flow-based adhesion assay. When gently agitated with platelets, freshly isolated from isofluorane-anaesthetised C57BL/6 mice, murine EPCs exhibited significantly greater binding compared to mature endothelial cells (ECs) or fibroblasts. This was illustrated by increased numbers of large cellular aggregates detected by Coulter Counter size distribution. For flow-based adhesion, platelets were immobilised on glass capillaries. When perfused through the capillary at a wall shear stress of 0.025 Pa, stationary adhesion of EPCs to platelets was observed at a much higher level compared to perfused ECs or fibroblasts. Through selective blockade of pathways involved in platelet activation and of intracellular adhesion molecules, we aim to define mechanisms responsible for EPC adhesion to platelets which may underlie attachment to the vessel wall *in vivo*.

[1] de Boer et al. (2006). *Arterioscler Thromb Vasc Biol* 26:1653-1659

[2] Langer et al. (2006). *Circ Res* 98(2):e2-10

[PP53]

MORPHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF TNP-470 ON THE REGENERATING VASCULATURE DURING WOUND HEALING IN THE MURINE *PANNICULUS CARNOSUS*

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TNP-470 was the first anti-angiogenic agent to undergo clinical trials in human tumours. The main complication of anti-angiogenic therapy for tumours is its unavoidable effect on physiological processes such as wound healing. Our aim was to quantify the effect of TNP-470 administration on longitudinal healing and vascular ingrowth in a murine wounding model. Following anaesthesia with hypnorm and hypnovel, dorsal window chambers were surgically fitted to mice and small skeletal muscle wounds created within the field of view (1). During the period of most active angiogenesis (up to day 9 post injury) wound closure is significantly delayed by TNP-470 (30mg/kg/2d sc). Both the extent of vessel sprouting and recovery of tissue perfusion were inhibited compared to vehicle-injected mice. Animals receiving TNP-470 present newly formed plexi which are more closely aligned with myofibrillar direction (by day 6 post injury) than those injected with vehicle alone (anisotropy index: 22 ± 8 [TNP] vs 4 ± 3 [Controls]: $p < 0.05$). Furthermore, TNP-470 reduces macromolecular flux across the vessel wall of blind-ended vessels at the leading edge of the advancing vascular plexus, as measured by *in vivo* Fluorescence Recovery After Photobleaching, over days 5-7 post injury. These findings show that during the wound healing response of *panniculus carnosus*, the administration of TNP-470 inhibits angiogenesis, deregulates plexus morphology and progressively reduces the leakiness of newly formed vessels.

1. Guerreiro-Lucas et al. (2008) *Microvasc Res* 76, 161-8.

[PP54]

ADAMTS-4 MAY BE A NOVEL ANTI-ANGIOGENIC MOLECULE

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Vascular endothelial growth factor (VEGF) is the most potent angiogenic factor stimulating endothelial cell proliferation, migration and tube formation and mediates angiogenesis by binding to receptor kinases VEGF-R1/R2 and neuropilins (NP1/2). A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-1 and -4 are two aggrecanases, and ADAMTS-1 is known to be anti-angiogenic. As ADAMTS-4 is closely related to ADAMTS-1 and is expressed by endothelial cells, this study aimed to investigate the potential role of ADAMTS-4 in angiogenesis by assessing its effects on tubule formation (Matrigel assay), migration (scratch assay) and VEGF induced VEGFR2 phosphorylation of human dermal microvascular endothelial cells (HuDMEC). Recombinant human (rh) ADAMTS-4 significantly inhibited the number of VEGF stimulated tubules formed on Matrigel ($p=0.0304$). Similarly ADAMTS-4 significantly inhibited migration of HuDMEC in both the absence ($p=0.0326$) and presence ($p=0.0240$) of VEGF. Western blotting analysis revealed that ADAMTS-4 also reduced VEGF stimulated VEGF-R2 phosphorylation. Work is currently underway to assess the effects of ADAMTS-4 on HuDMEC proliferation. These data suggest that ADAMTS-4 may be a novel anti-angiogenic factor which mediates its effects by modulating the VEGF-VEGF-R2 signalling pathway.

BIOPHYSICS, METHODOLOGY AND DEVELOPMENTAL TOOLS

[PP55]

FLUORESCENCE LYMPHOGRAPHY IN NUDE MICE TO STUDY LYMPHATIC CLEARANCE *IN VIVO*

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We previously showed that human lymphatic endothelial cells (LEC) injected into the skin of nude mice promote tumour growth towards these cell depots [1]. 21 days after LEC injection there was a statistically significant increase in the lymphatic vessel density (LVD) in the skin surrounding the cell injection site ($7.4 \pm 1.6 \text{mm}^{-2}$ mean \pm SEM) in comparison with normal skin away from the injection site ($2.5 \pm 0.2 \text{mm}^{-2}$). To determine if these additional vessels result in increased lymphatic function, we performed fluorescence lymphography on adult nude mice injected subcutaneously with 10^5 LEC, tattooing the injection site with Monastral Blue dye. At day 28, mice were injected intradermally into the interscapular region with $50 \mu\text{l}$ of 0.16% FITC-Dextran 150kDa and fluorescence was measured every hour, up to 6 hours after the injection, with an IVIS Lumina (Caliper Life Sciences). As a control, we injected mice with saline, and in a separate group culled mice immediately after the dye injection. In control mice, fluorescence decreased mono-exponentially with a half-life of 70min. LEC injected mice had a similar decay, with a half-life of 93min. There was no reduction in fluorescence in dead mice. These results suggest that directed tumour growth towards LEC depots is not due to increased lymphatic function. Moreover, this method of studying lymphatic clearance can be useful in investigating modifications of the lymphatic drainage of the skin.

[1] Shields *et al.* (2007). *Oncogene* 26:2997-3005

[PP56]

ON THE SPATIAL CHARACTERISTICS OF BLOOD VISCOSITY IN THE MICRO-SCALE

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In the present work the spatial variation of blood viscosity was examined *in-vitro* using modelling combined with shear rate fields estimated by a micro-PIV based technique in a plate-plate geometry with a gap of 30 microns. Blood samples at normal hematocrit levels, and in the presence of physiological red blood cell (RBC) aggregation intensity, were subjected to a simple shear flow. Blood flow velocity and microstructural characteristics were determined from images captured using a high speed camera. It was observed that RBC aggregation caused changes in the velocity field; aggregate and network formation lead to deviations from the expected flow. The anisotropic microstructure of blood was found to influence its mechanical properties in an analogous manner; the viscosity of blood estimated in different regions of the flow was found to vary significantly, both spatially and with relation to the overall effective viscosity. The findings suggest that for blood flows where partition of aggregates or breakdown of RBC network occurs, such as flows involving junctions and bifurcations, blood hemodynamics and effective hemorheological properties will be significantly affected by the spatial variation of viscosity.

[PP57]

DEVELOPMENT OF THE DORSAL SKIN-FOLD WINDOW CHAMBER IN MICE FOR THE ANALYSIS OF ANGIOGENIC GROWTH FACTORS

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Mature vasculature is required to effectively supply a tissue with its metabolic needs. A complex network of angiogenic growth factors directs blood vessel growth and maturation. This study developed and optimised an experimental system by which growth factors can be introduced into the murine dorsal skin-fold window chamber to study their role in vascular growth and maturation. The suitability of three materials (matrigel, polyether sponge, polyvinylpyrrolidone(PVP) hydrogel) as growth factor carriers in the window chamber was tested. Surgery was conducted under hypnorm/hypnovel anaesthesia. ELISA was used to measure growth factor release from hydrogels *in vitro*. Window chamber vasculature was monitored *in vivo* following growth factor carrier implantation via intravital microscopy then tissue fixed for immunohistochemical analysis of CD31 and α -smooth muscle actin. PVP hydrogel was most suitable for *in vivo* implantation, allowing control over growth factor release rate and protection from enzyme degradation. 0.5% crosslinker hydrogels were used to encapsulate VEGF120 or 164. VEGF164 implants increased vascular development particularly in areas immediate to the hydrogel, forming microvascular clusters. VEGF120 stimulated vascular extension/widening at a distance from the hydrogel. VEGF189 is under evaluation using this novel experimental system.

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[PP58]

MODELLING INERT GAS CLEARANCE FROM TISSUES BY THE MICROCIRCULATION

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All models of inert gas clearance from tissues by the circulation that are used to understand decompression after diving, recovery from anaesthesia and for interpreting washout estimations of tissue blood flow, assume simplifications in the interaction between convective and diffusive processes eg. that clearance is flow dependent and tissue gradients of concentration (partial pressure) are neglected. We have obtained numerical solutions without these assumptions for models where inert gas in a cylindrical tissue block is cleared by one or two parallel axial-directed vessels. So far we have used them to understand flow estimates based on tissue clearance. Effects of distance between the two vessels, variations in flow velocity and direction and blood-tissue partition coefficient of the inert gas have been investigated. The reciprocal of the clearance time constant ($1/\tau$) varied almost linearly with total volume flow in both single and co-current flow models. As expected, counter-current flow cleared the tissue more slowly but comparison with co-current flow revealed how large the difference could be. With equal flow velocities in the two vessels, changes in total flow in counter-current vessels gave changes in clearance rate that were $<1/5$ of those seen for the same changes in co-current flow. Intermediate states of counter-current flow (30% total flow in one direction, 70% in the other) reduced clearance proportionately indicating that changes in clearance accompanying changes in patterns of flow may be incorrectly interpreted as changes in total flow.

[PP59]

COMPARISON OF *IN-VIVO* AND *EX-VIVO* VASCULAR REACTIVITY IN MSK_{1/2} KNOCK-OUT MICE AS MEASURED BY LASER DOPPLER IMAGING AND WIRE MYOGRAPHY

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We developed a strain of mice that lack expression of 2 protein kinases, mitogen- and stress-activated protein kinase 1 (MSK1) and MSK2 that are important in inflammation and endothelial function. The purpose of this study was to examine vascular function of MSK_{1/2} knock-out (KO) mice *ex-vivo* and compare this to *in-vivo* microvascular responses. In-vivo skin microvascular responses to iontophoresis of acetylcholine and sodium nitroprusside were measured monthly using laser Doppler imaging (LDI). Vascular function was measured *ex-vivo* (at 6 months) in the tail artery by measuring relaxation to ACh and SNP using wire myography. There was a significant reduction in in-vivo microvascular responses to ACh over 6 months in KO mice compared to wild-type (WT) animals, no significant difference was seen in the maximal response to SNP. In the tail artery, max relaxation was significantly reduced in KO compared to WT and significantly correlated with in-vivo measurements at weeks 12 (P=0.005), 16 (P=0.002), 20 (P=0.012), 24 (P=0.004). There was a significant rightward shift in the dose-response curve to SNP for the KO mice fed an atherogenic diet, indicating decreased sensitivity in this group. We have shown that measuring microvascular function using LDI *in-vivo* is comparable to results in the tail artery examined *ex-vivo* by wire myography. Therefore our in-vivo microvascular function measurements are reflective of systemic vascular function in mice.

[PP60]

ATOMIC FORCE MICROSCOPY (AFM): A NOVEL TECHNIQUE FOR IDENTIFYING CANCER CELL & BONE MICROVASCULAR ADHESION MOLECULES *IN VITRO*

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Primary cancers such as prostate and breast often metastasise to the skeleton, with bone tumours being a major cause of death. The aim of this work is to characterise tumour cell adhesion molecules and correlate with their ability to interact the bone microvasculature using AFM. Human bone marrow endothelium (BME) cells were grown on tissue culture dishes. A single prostate (PC3) or breast (MDA-B02) cancer cell was coupled to the end of the AFM cantilever. Once baseline values for adhesion force were obtained, studies were repeated with functional blocking antibodies (20mg/ml) and the bisphosphonate, Zoledronic acid (Zol). Prostate: anti-ICAM-1, anti-VCAM-1, anti-P-selectin, anti- β 1. Breast: anti- α v β 3 and anti-Ep-CAM. There was a positive adhesion interaction between PC3 and BME cells (average adhesion events/curve 8.3 ± 1.8) which was reduced by anti-VCAM-1 (7.8 ± 1.7), anti- β 1 ($6.8 \pm 1.5^*$), anti-ICAM-1 ($6.9 \pm 1.6^*$), anti-P-selectin ($6.5 \pm 1.5^*$), blocking antibodies combined ($4.28 \pm 1.0^*$) and Zol ($4.5 \pm 1.8^*$). Positive adhesion interactions between MDA-B02 and BME cells (9.1 ± 1.0) were reduced by anti- α v β 3 ($5.8 \pm 1.4^*$), anti-Ep-CAM ($6.0 \pm 1.9^*$), blocking antibodies combined ($4.9 \pm 1.5^*$) and Zol ($4.3 \pm 0.7^*$). * $p < 0.05$. This study has identified possible adhesion molecules involved in the interaction of prostate and breast cancer cells to bone microvasculature using AFM, and is a novel, sensitive screening assay to quantify adhesive strength of tumour-BME cell interactions.

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[PP61]

COMPARISON OF MOOR VMS AND DRT4 FLUXIMETER, FOR THE ASSESSMENT OF SKIN MICROCIRCULATION

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Background: Laser Doppler techniques to measure cutaneous microcirculatory perfusion are increasingly utilised in research and clinical practice. We evaluated Moor instruments Vascular Monitoring System (VMS), a new device for assessing dynamic microcirculatory perfusion responses, compared to the current standard Moor Doppler and Resistive Temperature Monitor (DRT4). Methods: We measured microcirculatory reactive hyperaemia in response to a 4 minute arterial occlusion in 8 subjects using Moor VMS with automated cuff inflation/deflation on one randomly allocated foot, and Moor DRT4 with manual cuff inflation/deflation on the other leg using a time constant of 3.0 seconds for both devices. We compared resting flux, flux during occlusion, peak response, time to peak and time to baseline. All data were analysed using the VMS data software and statistical analysis carried out using Wilcoxon signed-rank test. Results: All parameters were comparable between the VMS and DRT4. Maximum peak response was 44.75(29.47-72.95) (median, 25th quartile and 75th quartile) for DRT4 vs. 41.05(21.5-85.57)AU for VMS; p=1.0 and time to peak was 22.5(9-40.5) vs. 18(10.5-36.5)seconds; p=0.45 respectively. Conclusion: With a sample size of 8, we were unable to detect a difference between the VMS and DRT4. This suggests that the VMS may be a suitable alternative to the DRT4, with the advantage that, due to ease of use of the automated protocol, the investigator may focus on the quality of data collection and on the volunteer.

[PP62]

SEPARATING THE EFFECTS OF HYDROSTATIC PRESSURE AND CELLULAR STRETCH: DEVELOPMENT OF AN *IN VITRO* MODEL SYSTEM

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Endothelial cells (ECs) are constantly subjected to haemodynamic forces, including pressure and stretch (strain). These Stimuli are known to influence ECs, modifying their morphology, intracellular signalling & gene expression. Most reported systems exposing EC to mechanical forces *in vitro* alter pressure and stretch simultaneously, making it impossible to distinguish the two potentially independent stimuli. This distinction is particularly relevant when examining the interaction of haemodynamic forces on microvascular ECs, which are exposed to low hydrostatic pressure but significant strains. This research aims to create an *in vitro* system that can independently examine the effects of pressure and stretch (commensurate with the microcirculation) on EC function. Within the model the cells were seeded on the luminal surface of a compliant tube. The cell lined tube was mounted in a sealed outer chamber. Cyclical strain (1Hz) was generated by applying a negative pressure to the sealed outer chamber via a syringe drive. Luminal pressure was controlled by two hydrostatic pressure heads. Validation experiments show that pressure (0,15,25,35 mmHg) and stretch (0,5,10%) were independently controlled and maintained over 48 hours.

[PP63]

UTERINE SPIRAL ARTERY REMODELLING IN NORMAL AND TYPE 1 DIABETIC PREGNANCIES: IMMUNOCYTOCHEMICAL AND DOPPLER FLOW STUDY

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In early pregnancy, cytotrophoblast cells invade uterine spiral arteries replacing both the endothelial and smooth muscle cells. The resultant high flow, low resistance circulation is thought to be essential for optimal placental perfusion. Placental insufficiency in pre-eclampsia is attributed to insufficient remodelling and correlates with the impedance to uterine arterial (UA) flow on Doppler studies. Spiral artery remodelling in Type 1 diabetes (T1D) pregnancies is not established. UA Dopplers were performed, at 22-24 weeks, on patients with T1D (18) and normal pregnancies (45). Biopsies of decidua basalis were taken from both groups (N=3). The uterine spiral arteries therein were immunostained with the endothelial cell marker vascular endothelial-cadherin (VE-cadherin) and the trophoblast cell marker cytokeratin-7. Vascular profiles were classified as fully remodelled (cytokeratin-7 staining alone), partially remodelled (cytokeratin-7 and VE-cadherin) or unaltered (VE-cadherin alone). 61% of vessels in the normal group compared to 16% in T1D showed remodelling ($p < 0.001$). Resistance index (RI) of UA, between the groups showed no significant differences (LUA $p \geq 0.16$; RUA $p \geq 0.56$). Our data suggests that UA Doppler may not be sensitive enough to diagnose cellular remodelling of spiral arteries in T1D pregnancies. The necessity of all spiral arteries needing remodelling for optimal placental perfusion also requires questioning.

[PP64]

MULTIMODAL MULIPHOTON IMAGING OF VASCULAR WALLS

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Multimodal non-linear microscopy can be used to image the vasculature in order to visualise fibrous proteins and cell membranes without the need for sectioning or staining. The entire wall thickness can be imaged, using two photon fluorescence to image the elastin, second harmonic generation to image the collagen, and coherent anti-Stokes Raman scattering (CARS) to image the cells.

Bovine mesenteric collecting lymphatic vessels were mounted in a vessel bath and imaged under different luminal pressures (0-30cmH₂O pressure head), and longitudinal tensions. Human omental arteriole samples and equine renal artery were also sampled. In the lymphatic vessels large collagen fibre (15-25µm diameter) bundles were present in the inner media and small collagen fibres (2-5µm diameter) were distributed throughout the wall. The responses to longitudinal tension and luminal pressure indicated that the larger fibres resist the longitudinal strain and the smaller oppose pressure forces. Individual elastin fibres were of uniform thickness (1-3µm) and interwove amongst themselves and between the collagen fibres. After comparison with blood vessel structure, we speculate that the network's main function is to maintain the organisation of collagen bundles during contractile recovery. In order to test this and related hypotheses the data stacks have been digitised using Simpleware software [1] and will form the basis of structurally-based finite element models of wall micromechanics.

[1] Tabor, G., et al., Eng App Comp Fluid Mech, 2007. 1(2): p. 126-135.

NEUROBIOLOGY

[PP65]

BLOOD-BRAIN BARRIER DISRUPTION IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an auto-immune disease of the central nervous system (CNS) induced by auto-reactive T-cells that induce damage to multiple components of the CNS, including neurons, axons and myelin sheaths. A significant effect of this inflammatory response is breakdown of the blood-brain barrier (BBB). Changes in BBB permeability have been noted clinically through gadolinium enhancement of early MS lesions in MRI however little is known about the relationship of permeability changes to the formation of new blood vessels in the tissue as the result of inflammation, a process known as angiogenesis. To model BBB changes in MS, the animal model, experimental allergic encephalomyelitis (EAE) was induced in 6-8 week old female C57BL/6 mice by immunization with myelin oligodendrocyte glycoprotein peptide (MOG, amino acids 33-55) and Complete Freund's adjuvant. Animals were sacrificed at varying time points throughout disease progression and lumbar spinal cords assessed for changes in permeability using a novel imaging technique. There was a significant increase in BBB permeability in the lumbar cord and cerebellum that preceded the development of clinical score. Permeability remained at an elevated but stabilized level throughout disease progression. Current experiments are exploring whether these permeability changes occur in relation to angiogenesis. If angiogenesis occurs in EAE, we want to see if it is responsible for driving other aspects of the disease, and determine if blocking both angiogenesis and the associated BBB disruption can improve disease outcomes.

[PP66]

ASTROCYTE REMODELLING DURING DEVELOPMENT OF THE MURINE RETINAL VASCULAR PLEXUS

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In neonatal mice, the superficial retinal vascular plexus (RVP) forms on an astrocyte layer which migrates in advance of the endothelial cells. We investigated RVP remodelling in C57BL6/J wild-type and transgenic littermates over expressing lens specific VEGF-A₁₈₈ [1]. Isolectin-B4 (endothelium) and Pax2 (astrocyte) wholemounted retina were imaged with confocal microscopy. Significant remodelling events occur in the superficial RVP between P5 and P20, characterised by decreased vessel and branch point density ($p \leq 0.0001$), and increased mean capillary segment length ($p \leq 0.0008$). Parallel with RVP remodelling, a ten-fold reduction in astrocyte density is observed between P3 and P20 ($p \leq 0.0001$). Activated caspase-3 immunohistochemistry reveals endothelial capillary segment and astrocyte apoptosis in a progressive sequence from the optic chiasm towards the retinal periphery. At P3, astrocyte density in wild-type and VEGF₁₈₈ mice is similar; however, P20 VEGF₁₈₈ retinas have significantly more astrocytes ($p \leq 0.0001$). No significant difference in the branch point or vessel density of the superficial or deep RVP are observed between P20 wild-type and VEGF₁₈₈ mice, though mean RVP capillary segment length is shorter in VEGF₁₈₈ mice ($p = 0.0022$). These results indicate that the events of astrocyte apoptosis and RVP remodeling are co-ordinated during retinal post-natal development. We aim to further characterize the role of astrocytes during RVP remodelling using VEGF₁₈₈ mice as a model.

[1] Mitchell *et al*, *Angiogenesis* 2006;9:209-24.

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[PP67]

DIFFERENTIAL SECRETION OF VEGF ISOFORMS BY NEUROBLASTOMA CELL LINES

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VEGF is a key mediator of angiogenesis and is up-regulated in a variety of tumours. An endogenous family of anti-angiogenic isoforms, VEGF_{xxx}b, has been identified in normal, non-angiogenic tissues, and in contrast with the angiogenic VEGF_{xxx} isoforms, is down-regulated in epithelial tumours including colorectal and prostate carcinoma. This is the first study of the anti-angiogenic variant VEGF₁₆₅b in a malignant tumour of childhood, neuroblastoma (NB). Five NB cell lines, BE(2)-C, IMR-32, SH-IN, SH-SY5Y and SH-EP, with different tumorigenic potential and *MYCN* amplification status, were evaluated for the expression of total VEGF and VEGF_{xxx}b isoforms at protein level. ELISA assays on total cell lysate and a modified cell ELISA, were used to measure the levels of cellular and secreted VEGF isoforms, respectively. HEK293 cells were used as a positive control for the expression of VEGF_{xxx}b. All NB cell lines expressed VEGF and VEGF_{xxx}b isoforms. The *MYCN* amplified cell lines BE(2)-C and IMR-32 showed the lowest ratio of VEGF_{xxx}b/total VEGF (54±8% and 57±8% of HEK293 cells, respectively). On the other hand, the non-tumorigenic cell line SH-EP showed the highest levels of VEGF_{xxx}b. After 20 hours incubation, all NB cell lines secreted VEGF at a level between 1 and 20fg/cell. In contrast, VEGF_{xxx}b secretion was 1-2 orders of magnitude lower, varying between 0.2 and 0.6fg/cell. Of all NB cells only SHIN and SH-EP secreted more than 5% of its VEGF as VEGF₁₆₅b (18±8% and 10±6), whereas all the detectable VEGF secreted from HEK293 cells was VEGF_{xxx}b.

[PP68]

VEGF-A_{165b}-MEDIATED HIPPOCAMPAL NEUROPROTECTION IN VITRO IS DEPENDENT ON THE ACTIVITY OF VEGFR2 AND P42/44 MAPK SIGNALING

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The VEGF-A group comprises two distinct families due to alternative splicing of exon 8, the pro-angiogenic family VEGF-A_{xxx} and the anti-angiogenic family, VEGF-A_{xxx}b. VEGF-A₁₆₅ is neuroprotective for a large number of neuronal cell types against a variety of insults. To identify if VEGF-A_{165b} is also neuroprotective, mouse hippocampal neurons were dissected, dissociated and plated onto coverslips. After 7 days in culture the neurons were treated with 3 mM L-glutamic acid (Glu) for 24 hours to induce excitotoxicity, followed by the incubation with a live-dead kit and a Hoechst nuclear stain. Glu-induced excitotoxicity was calculated as the percentage of dead cells after treatment. Co-incubation with 10 nM VEGF-A_{165b} decreased Glu excitotoxicity by 46±7% (p<0.001). This VEGF-A_{165b}-mediated neuroprotection could be blocked by both inhibition of the VEGF receptor, VEGFR2 with 10 nM ZM323881 (p<0.01), and receptor tyrosine kinase activity with 200 nM PTK787 (p<0.05). Inhibition of VEGFR1 with 200 nM SU5416 had no effect on VEGF-A_{165b}-mediated neuroprotection (p>0.05). Treatment with 15 µM p42/44 MAPK inhibitor PD98059 also significantly blocked the neuroprotection (p<0.01) indicating that receptor signalling is required for the neuronal survival in response to VEGF-A_{165b}. Work to further elucidate the direct neuroprotective property of VEGF-A_{165b} may uncover potential roles for the protein in the therapy of neurodegenerative diseases.

Travel Information

Address

Peninsula Medical School, University of Exeter, St Luke's Campus,
Magdalen Road, Exeter, EX1 2LU

Directions to the St Luke's Campus

By Car

The M4/M5 links Exeter directly to London, the Midlands, South Wales and the North including Scotland.

From the M5

- Leave the M5 at Junction 29, signed Exeter Airport.
- At the traffic lights at the end of the motorway off slip road, turn right onto the B3015, signed City Centre. Go straight over at the first roundabout onto Honiton Road; Honiton Road Park & Ride will be on your left. Pass under the railway bridge and go straight over at the roundabout.
- At the traffic lights fork right, signed Heavitree/City Centre. Stay on this road until it becomes Fore Street (Heavitree) and you reach the shopping parade at Heavitree. At the traffic lights at the top of the hill, just after the Catholic Church on your right, fork left into Magdalen Road.
- Go straight over at the traffic lights, signed University (St Luke's). The St Luke's Campus, Magdalen Road entrance is approximately 500 metres on the right.

Parking

Delegates staying on campus will receive a parking permit if required. Non-residents can purchase a daily parking permit.

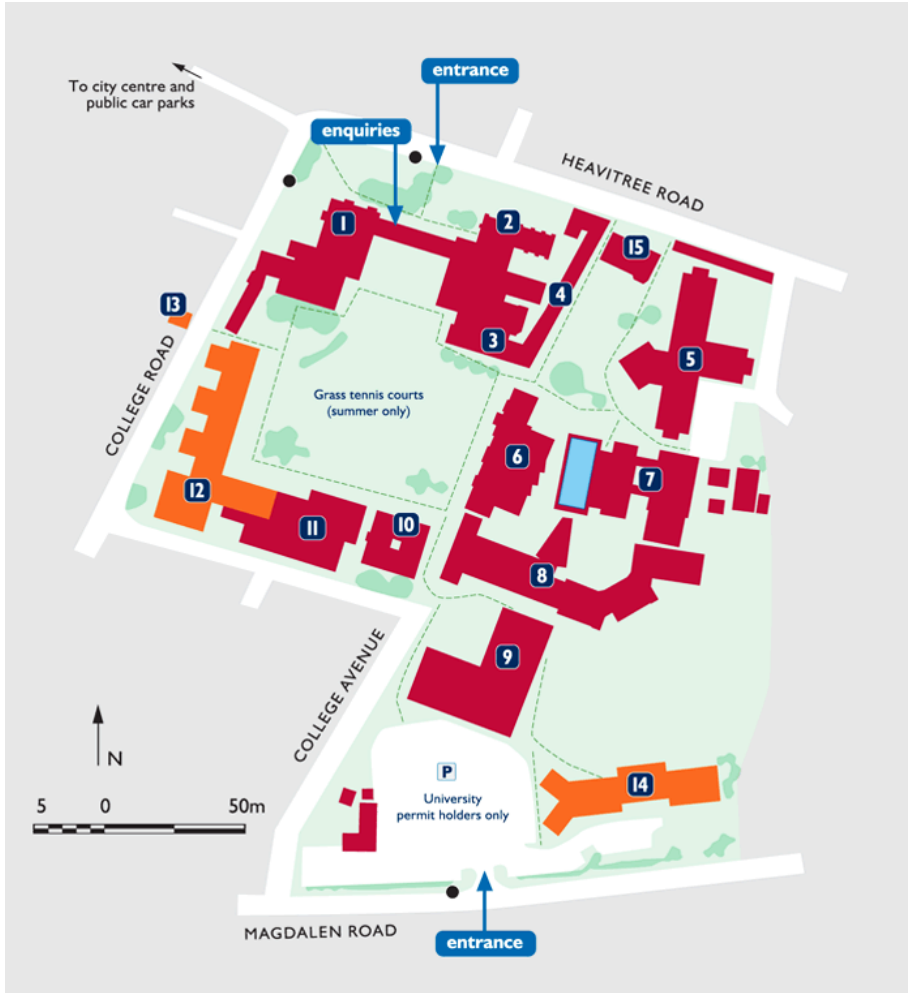
By Rail

Exeter has two railway stations - Exeter St. David's (main station) and Exeter Central. Exeter Central is approximately 20 minutes walk from St Luke's and taxis are available from both. Further information on planning your route can be found at <http://www.nationalrail.co.uk/> .

By Plane

Direct flights operate into Exeter from Glasgow, Edinburgh, Belfast, Dublin, Newcastle, Jersey, Guernsey, Alicante, Malaga and Brest-Brittany. Further information is available from <http://www.exeter-airport.co.uk>.

St Luke's Campus



- 1 North Cloisters (Lecture Theatre; NC12)
- 7 Sports Centre (registration, exhibition, posters, refreshments)
- 8 Peninsula Medical School
- 11 Cloisters Restaurant, South Cloisters
- 9 Richards Building
- 6 Highton, Library

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