atherosclerotic plaques. This study aims at visualising and characterising atherosclerosis using targeted ultrasmall superparamagnetic particles of iron oxide (USPIO) as an MRI probe for detecting inflamed endothelial cells and inflamed atherosclerotic plaques.

Method The in vitro study consists of detection and characterisation of inflammatory markers on activated endothelial cells by immunocytochemistry and anti-E-selectin antibody-conjugated USPIO. The ex vivo stage involves characterisation of inflammatory markers on human atherosclerotic plaques.

Results We have established an in vitro cellular model of endothelial inflammation induced with tumour necrosis factor α. We have confirmed the inflammation of endothelial cells with both immunocytochemistry and MRI. We can also image the inflammation of human atherosclerotic plaques by ex vivo MRI.

Conclusion We successfully developed an in vitro model to detect and characterise inflamed endothelial cells by immunocytochemistry and MRI. We can also image the inflammation of human atherosclerotic plaques by ex vivo MRI. This will allow us to develop agents and protocols for imaging vascular inflammation in atherosclerosis in the future. This pilot study will form the basis for a translational study to provide clinicians with a novel tool for in vivo assessment of atherosclerosis.

BAS/BSCR20 THE ROLE OF A GAB1—TRIBBLES 2 INTERACTION IN PHOSPHOINOSITIDE-3-KINASE, AKT/PKB CASCADE REGULATION AND CELLULAR MORPHOLOGY

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Apoptosis is a key event in atherosclerotic plaque formation. The phosphoinositide-3-kinase (PI3K) cascade is involved in many cellular activities in plaques, such as migration and cell survival. Our previous data have identified an interaction between Tribbles 2 (Trb2) and signalling adaptor molecule Grb2 associated binder protein (Gab1). The functional consequences of this interaction are unknown. Gab1 interacts with the p85 domain of PI3K to mediate downstream activation of the Akt/PKB anti-apoptotic signalling pathway. We examined whether tribbles, a new family of signalling regulators, link with PI3K to control activation of Akt and potentially inhibit apoptosis.

HEK293 cells were transfected with Trb2 and mutant or wt Gab1 and formation of Trb2/Gab1 complexes was quantified using a yellow fluorescent protein-based protein fragment complementation assay. We show that overexpression of the PI3Kδ and β (catalytic) and the PI3Kα (regulatory) subunits leads to an increase in Trb2—Gab1 interaction (relative binding intensity 2.48%± SEM vs 3.9%± SEM, $p<0.05$, and 2.52%± SEM vs 5.3%± SEM, respectively). These data suggest that Trb2/Gab1 binding is modulated via a PI3K dependent feedback loop and raise the possibility that that Trb2 may act as a co-regulator of Gab1. In addition, we show that Gab1 has an effect on cell morphology upon PI3K cascade activation, and this morphological consequence is potentiated further in the presence of Trb2. These findings in turn suggest that the Gab1—Trb2 interaction may participate in controlling cell survival and morphology and potentially, atherosclerotic plaque development or rupture.

BAS/BSCR21 AKT PROTECTS THE HEART BY PROMOTING MITOCHONDRIAL FUSION

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Background Mitochondria change their morphology by undergoing 'fusion' and 'fission' to generate elongated and fragmented mitochondria, respectively. We hypothesised that inducing mitochondrial fusion protects the heart against ischaemia-reperfusion injury (IRI), and that this mechanism underlies the cardioprotection elicited by the pro-survival kinase, Akt.

Methods/results Inducing mitochondrial fusion in HL-1 cells (a cardiac cell line), using mitochondrial fusion proteins and a pharmacological inhibitor of a mitochondrial fission protein (called mdivi-1) delayed the opening of the mitochondrial permeability transition pore (mPTP, a critical mediator of IRI) and reduced cell death following IRI. Overexpressing Akt induced mitochondrial elongation (49.0±5.8% control vs 72.8±5.0% Akt; N=4 experiments:*$p<0.05$), delayed mPTP opening (twofold; N=4 experiments: $p<0.05$); and reduced cell death following IRI (64.9±5.6% control vs 34.2±1.2% Akt; N=4 experiments:*$p<0.05$). Treatment with the cytokine, erythropoietin (EPO, 10 U/ml), also induced mitochondrial elongation (30.0±3.5% with control vs 67.0±3.4% for EPO; N=4 experiments:*$p<0.05$), delayed mPTP opening (twofold; N=4 experiments: $p<0.05$); and reduced cell death following IRI (43.1±2.7% control vs 17.0±2.7% EPO; N=4 experiments:*$p<0.05$). Finally, elongated mitochondria extending 4—6 μm in length were observed in adult rodent hearts using electron and confocal microscopy. Treatment with mdivi-1 increased the number of elongated mitochondria and protected against IRI, as shown by reduced cell death in adult cardiomyocytes following IRI, and reduced myocardial infarct size in the in vivo murine heart.

Conclusions Inducing mitochondrial fusion protects the heart against IRI by delaying mPTP opening. Akt activation also promotes mitochondrial fusion, delays mPTP opening and protects against IRI. These results suggest that modulating mitochondrial morphology may be a novel strategy for cardioprotection.

BAS/BSCR22 TIME SERIES ANALYSIS OF ACUTE CORONARY SYNDROME FROM PERIPHERAL WHOLE BLOOD USING AFFYMETRIX GENECHIP ARRAYS

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Introduction Acute coronary syndrome (ACS) is the cause of over 114 000 UK hospital admissions[1] and a cost to the NHS of over £3.9 billion every year.[2] Advances in microarray technology allow a detailed understanding of genome-wide expression profiles of pathological processes. We hypothesised that analysis of ACS, at the time of an acute event and throughout recovery, would provide insight into pathology, as well as identify genes as potential drug targets and both diagnostic and prognostic markers.

Methods 50 patients presenting with chest pain consistent with ACS were recruited within 48 h of admission. 3 ml of peripheral whole blood was collected using Tempus RNA tubes at days 1, 3, 7, 30 and 90. Total RNA was extracted, cleared of globin mRNA and arrayed using Affymetrix HG_U133 plusv.2 GeneChips. Data were analysed using open source software PUMA.

Results We used principal component analysis (PCA) to visualise the data. With clinical information incorporated, it was found that the data discriminated between patients, putting them into troponin-positive and troponin-negative groups across all time points. Hierarchical clustering, comparing the expression profiles between groups, identified different clusters of genes that increased in expression over time in the troponin-positive group. Pathway analysis of the clusters showed overexpression of Rho GTPase cytoskeletal, endothelin signalling, integrin signalling, G-protein signalling and inflammation-mediated pathways.

Conclusions Microarray analysis identified expression differences between troponin-positive and troponin-negative patients over time. Specific biological pathways possibly showing the late effects of acute events can be used to discover biomarkers of coronary heart disease.

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BAS/BSCR23 APOCYNIN TREATMENT REDUCES HIGH-FAT DIET-INDUCED OBESITY AND HYPERTENSION BUT HAS NO SIGNIFICANT EFFECT ON HYPERGLYCAEMIA

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Dietary obesity is associated with insulin resistance and cardiovascular oxidative stress. Apocynin has traditionally been regarded as an inhibitor of NADPH oxidase but recently it was reported to be predominately an antioxidant in the vascular system. In this study we examined the antioxidative stress effect of apocynin on high-fat diet (HFD)-induced metabolic disorders and endothelium dysfunction. Mice (C57/BL6 at 6–7 month of age, n=7 per group) were fed with a HFD (44% fat) or normal chow diet (12% fat) for 15 weeks. The treatment group was supplied with apocynin (5 mM) dissolved in drinking water and the control group was supplied with vehicle. Compared with chow diet, a HFD significantly increased the body weight (~35%), the systolic blood pressure (BP, 13%) and the levels of fasting blood glucose (46%). Apocynin treatment significantly attenuated the HFD-induced obesity (44.1%±2.96 vs 37.5%±2.43 g) and the high BP (136.7%±7.9 vs 118.4%±5.3 mm Hg), but had no significant effect on blood glucose levels (8.74%±1.62 vs 8.13%±1.68 mmol/l). Compared with a chow diet, HFD significantly impaired the endothelium-dependent vessel relaxation to acetylcholine as examined by an organ bath, and this was reversed to control levels by adding tiron, which is a cell membrane permeable superoxide scavenger. Apocynin treatment preserved endothelium-dependent vessel relaxation to acetylcholine in the HFD group. In conclusion, antioxidant treatment with apocynin attenuated the HFD-induced increases in body weight; blood pressure and preserved the endothelium function. However, apocynin had no effect on HFD-induced increase in fasting blood glucose levels.

BAS/BSCR24 SPIRONOLACTONE REVERSES THE ADVERSE EFFECTS OF ALDOSTERONE AND HYPOXIA ON ADIPOSE TISSUE

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We tested the hypothesis that aldosterone causes a loss of the normal anticontractile function of healthy fat via a hypoxia-related pathway, which can be rescued using spironolactone. Healthy rat mesenteric arteries (~250%μM diameter) and perivascular fat were investigated using wire myography and Perl's Prussian blue staining for activated macrophages. The effects of aldosterone%±spironolactone were assessed after incubation for 10 min and 3 h, and experimental hypoxia (95% N_2/5% CO_2) for 2.5 h. Contractile responses were calculated as a percentage of KCl contraction and expressed as mean±SEM. Macrophage activation was expressed semiquantitatively and expressed as macrophage abundance values (MAV). The anticontractile capacity of healthy fat was lost upon incubation with aldosterone (5 nM) (fat: 90±4% n=36, fat+10 min aldosterone: 165±5% n=25, fat+3 h aldosterone 172%±12% n=7) and was associated with an increase in activated macrophages (immediately fixed: 2.2±0.5% n=5 vs 10 min aldosterone: 3.8±0.4% n=5, p=NS; immediately fixed: 2.2±0.5% n=5 vs 3 h aldosterone: 4.7±0.3% n=5, p=0.0313). Spironolactone (10%μM) restored anticontractile activity after incubation for 3 h only (3 h:111±4% $p<0.05$, n=5) and caused a significant reduction in macrophage activation (3.0±1.0%, n=5). As for aldosterone, hypoxia caused an increase in contractility (149±17% n=15) and macrophage activation (5.5±0.5% n=5), which was reversed upon incubation with spironolactone (contractility: 120±12% n=5;MAV: 3.3±0.8%, n=3, p=0.500). Aldosterone ameliorates the anticontractile capacity of healthy fat by a common pathway to hypoxia, which correlates with an increase in the number of activated macrophages within adipose tissue. Spironolactone can restore the effect of hypoxia on contractility and macrophage activation in the absence of aldosterone.

BAS/BSCR25 IMPORTANCE OF INTERACTION BETWEEN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-α AND NADPH OXIDASES IN CARDIAC HYPERTROPHY

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Peroxisome proliferator-activated receptor-α (PPAR-α) and NADPH oxidases are known individually to have essential roles in left ventricular hypertrophy (LVH). However, the potential importance of interaction between the two in overall regulation of the hypertrophic phenotype is unclear. Here Nox2−/−, PPAR-α−/− and matched wild-type (WT) mice (n=8) underwent thoracic aortic constriction (TAC) or sham surgery and were studied 7 days later. No differences in basal contractile function (echocardiography) or LVH were observed. However, increased mRNA expression (real-time RT-PCR) of PPAR-α in Nox2−/− (1.49%±0.08 vs WT, 1.04%±0.11 arbitrary units; $p<0.05$) and of Nox2 in PPAR-α−/− mice (3.61%±1.21 vs WT, 0.95%±0.12 arbitrary units; $p<0.05$), together with increases in NADPH oxidase activity (lucigenin-enhanced chemiluminescence: PPAR-α−/−, 6.81%±0.94 vs WT, 3.49%±0.35 RLU; $p<0.05$), indicated co-regulation of myocardial Nox2/PPAR-α. As expected, TAC-induced LVH was significantly increased in PPAR-α−/− versus WT mice, but similar in Nox2−/− mice. Decreases in fractional shortening in WT mice (−16%±3%) were augmented in PPAR-α−/− and attenuated in Nox2−/− mice after TAC (−28%±4 and −10%±3; $p<0.05$ vs WT). PPAR-α mRNA expression was increased in WT, but not Nox2−/− mice (52%±11 vs −17%±11; $p<0.05$), while Nox2 mRNA expression remained elevated (1.33%±0.22 vs 0.69%±0.16 arbitrary units; $p<0.05$) in PPAR-α−/− versus WT mice after TAC. NADPH oxidase activity was significantly increased in TAC WT, but not PPAR-α−/− or Nox2−/− mice, although absolute levels in PPAR-α−/− remained elevated compared with WT sham. These data indicate co-dependence of PPAR-α and NADPH oxidases in the setting of pressure-overload LVH, although the underlying mechanisms are clearly complex.