201 FOCAL ADHESION PROTEIN AND CYTOSKELETAL REMODELLING IN NORADRENALINE AND ENDOTHELIN-1 STIMULATED VASCULAR SMOOTH MUSCLE CELLS

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Background Focal adhesions (FA) are dynamic transmembrane protein complexes serving as mediators of signalling between the extracellular matrix (ECM) and the actin cytoskeleton (CK). Remodelling of FAs and the CK play key roles in some of the pathological functions of vascular smooth muscle cells (VSMC) such as proliferation, migration and contraction. Focal adhesion proteins (FAPs), constituents of FAs, indirectly dictate some VSMC functions. The FAPs, paxillin, Hic-5 and vinculinâ€™s presence within FAs is well established in VSMCs, however, their behaviour in response to biomechanical and vasoconstrictor stimuli are not. Endogenous vasoconstrictors such as Endothelin-1 (ET-1) and Noradrenaline (NA), alongside changes in ECM have individually promoted changes in the VSMC CK and FA arrangement. To contextualise these cytoskeletal changes and their consequences on vascular function, more information is needed about FAP localisation and CK arrangement in response to vasoactive stimulation alongside ECM changes.
Aims To investigate actin CK remodelling and the localisation of Hic-5, vinculin and paxillin in response to changes in substrate composition and contractile stimulation.
Methods Rat VSMCS (RVSMCs) were cultured on glass or type I collagen-coated glass and were stimulated with 15Î¼M NA, 100 nM ET-1 or remained unstimulated. Cells were stained for actin filaments and either Hic-5, vinculin or paxillin. Changes in CK arrangement were visually assessed. The punctate regions of FAPs were quantified using image analysis software.
Results When cultured on glass, Hic-5 and paxillin occupied punctate regions within RVSMC; vinculin was diffusely spread throughout the cell. The punctate regions were principally localised to the ends of actin stress fibres. Compared to unstimulated RVSMCs (punctate region mean area, 1.043Î¼mÂ²), NA caused a reduction in paxillin punctate region area of RVSMCs cultured on both glass (0.5548Î¼mÂ²; $P < 0.0001$) and collagen (0.7060Î¼mÂ²; $P < 0.001$). NA also induced cytosolic dissemination of Hic-5 without affecting punctate region area. Vinculin localisation did not change in response to NA ET-1 or collagen. ET-1 did not induce changes in paxillin or Hic-5 localisation or CK arrangement. RVSMCs cultured on glass showed a peripheral arrangement of the CK within the cell compared to those cultured on collagen, irrespective of stimulation.
Conclusion The study demonstrates that NA selectively regulates FAP localisation for paxillin and Hic-5, but not vinculin. As ET-1 does not regulate FAP localisation; these results indicate that individual FA remodelling is agonist specific within VSMCs. Furthermore, ECM composition is vital in CK reorganisation in a manner independent of NA-induced FA remodelling. Accordingly, actin CK and FA reorganisation in response to altered ECM composition or vasoconstrictors may contribute to vascular remodelling in cardiovascular disease.

202 AMPK ACTIVATION PARTIALLY RESTORES THE ANTI-CONTRACTILE EFFECT OF PERIVASCULAR ADIPOSE TISSUE IN FEMALE OFFSPRING OF OBESE RATS

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Introduction Maternal obesity programmes offspring to develop obesity and associated cardiovascular disease. Perivascular adipose tissue (PVAT) exerts an anti-contractile effect in healthy blood vessels; an effect lost in male offspring of obese dams. However, the mechanism by which maternal obesity programmes PVAT dysfunction in offspring remains unknown.
Methods Six week old female Sprague-Dawley rats were fed a control (10% fat) or 45% fat obesogenic diet (HFD) for 12 weeks before mating; during pregnancy and lactation. At weaning, female offspring were provided with the control diet until sacrifice at either 12 (12 wo) or 24 (24 wo) weeks of age. PVAT-denuded mesenteric arteries from pups, with or without exogenous PVAT, were mounted on a wire myograph and concentration-response curves were constructed to thromboxane A_2 receptor agonist U46619 (10 nM-3 μM) ± 10 μM A769662, an activator of AMP-activated protein kinase (AMPK) ± glucosamine (an O-GlcNAcylator). Western blotting was used to asses protein expression in PVAT stimulated with or without glucosamine.
Results Body weight, insulin levels and blood pressure were increased in HFD dams and their 12wo and 24 wo offspring compared to age-matched controls. Without PVAT, vessel contractions to U46619 were reduced in 12 wo offspring of HFD dams, effects mimicked in control arteries by pre-incubation with 10 mM glucosamine. PVAT from control, but not from HFD offspring, exerted an anti-contractile effect on the corresponding PVAT-denuded arteries at both ages. Pre-incubation of control PVAT with glucosamine diminished the anti-contractile effect at both ages. PVAT from HFD offspring pre-incubated with glucosamine had no effect on PVAT-denuded vessels but simultaneous AMPK activation within PVAT partially restored anti-contractile capability at both ages. Protein O-GlcNAcylation expression was increased in HFD PVAT and control PVAT incubated with glucosamine, whereas AMPK expression was decreased.
Conclusions The enhanced protein O-GlcNAcylation and decreased AMPK expression in HFD PVAT may underlie the reduced anti-contractile effect of PVAT in female offspring of obese dams. Nevertheless, simultaneous AMPK activation within HFD PVAT partially restored the anti-contractile effects of PVAT.

203 ELN GENE: UKGTN SERVICE FOR SVAS AND CUTIS LAXA. COPY NUMBER VARIANTS (CNVS) ARE A COMMON CAUSE OF DISEASE

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Pathogenic *ELN* gene mutations (*ELN*, MIM#130160) cause AD Supravalvular Aortic Stenosis (SVAS) a congenital narrowing of the ascending aorta, and Cutis Laxa (CL) characterised by inelastic, loose-hanging skin. Variable phenotype and penetrance is apparent. Pathogenic *ELN* variants result in loss of function and include frameshift (most common), nonsense, splice site and missense variants. The well characterised contiguous gene deletion syndrome, Williams-Beuren syndrome includes SVAS and encompasses at least 114kb on 7q11.23 including the *ELN* gene; however, there are only 5 case reports of CNVs within *ELN* (single or multiple exons).

Bristol Genetics Laboratory provides a UKGTN approved service for *ELN* gene sequencing (33 coding exons). In three years, 52 UK and foreign patients with SVAS, CL or features such as pulmonary artery stenosis and aortic dilation have been tested. 18/52 (34%) patients were heterozygous for a likely pathogenic variant including frameshift (6), nonsense (4), splice (4), and missense (4). 12 of these cases were novel variants, 5 are supported by segregation analysis and 1 is sporadic. The remaining novel variants are classed as possibly pathogenic as they are phenotypically compatible.

12/35 patients negative on sequencing have so far been screened for CNVs by MLPA (MRC Holland) covering the Williams-Beuren syndrome region, including 10 exons of the *ELN* gene (1, 3, 4, 6, 9, 16, 20, 26, 27 and 33) and in addition a bespoke MLPA assay including probes for exons 28 to 30, 32 and 3'UTR.

4/12 (33%) patients have a heterozygous deletion within the *ELN*gene. A mother and daughter with pulmonary stenosis and an extended family history have a deletion spanning exons 30 to 33. This deletion was also identified in another patient with SVAS and arteriopathy. A deletion of the 5' end of the gene, involving at least exon 1 (but not exon 3) was identified in an infant with SVAS and pulmonary branch stenosis, and a deletion involving the entire coding region of the *ELN* gene and at least the first two exons of the adjacent 3' gene *LIMK1* was detected in a neonate who died at 2 months with SVAS, pulmonary stenosis and mild hypoplasia with PDA. The deletion was detected in this patient's father who consequentially was found to have an aortic regurgitation and in a subsequent pregnancy of this family which was lost at 31 weeks with pulmonary stenosis and significant aortic stenosis

MLPA analysis has enhanced the clinical utility of this service giving an increased diagnostic yield in patients with SVAS and CL and related presentations.

204 GLYCOMIMETICS; A NOVEL CLASS OF DRUGS TO PROTECT AGAINST FREE FATTY ACID-INDUCED ENDOTHELIAL DYSFUNCTION

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Background Endothelial dysfunction is a key player in cardiovascular disease (CVD) complications and novel drugs are required to treat this pathological process. Glycosaminoglycans (GAGs) are key molecules that regulate signalling in many biological processes and drugs that mimic their structure could be a novel source of therapeutics to target specific CVD pathways.

Purpose We have synthesised a set of four glycomimetic compounds and our objective was to determine whether they could activate protective pathways in endothelial cells subjected to fatty acid-induced endothelial dysfunction.

Methods Glycomimetics, C1-C4, were synthesised by the stepwise transformation of 2,5-dihydroxybenzoic acid to a range of 2,5-substituted benzoic acid derivatives, incorporating the key sulphate groups to mimic heparan sulphate. Human Umbilical Vein Endothelial Cells (HUVECs) were treated with glycomimetics (1ÂμM) in the presence or absence of the free fatty acid, palmitate. DAF-2 and H_2DCF-DA assays were used to determine NO and reactive oxygen species (ROS) production, respectively. Lipid peroxidation colorimetric and antioxidant enzyme activity ssays were also carried out. RT-PCR and western blotting were utilised to measure Akt, eNOS, Nrf-2, NQO-1 and HO-1 expression. Endothelial function was determined *ex vivo* using acetylcholine-induced endothelium-dependent relaxation in mouse thoracic aortic rings by wire myography.

Results All four glycomimetics protected against palmitate-induced oxidative stress and enhanced NO production *in vitro* via upregulation of Akt/eNOS signalling, activation of the Nrf2/ARE pathway and down-regulation of ROS-induced lipid peroxidation. Under palmitate-induced oxidative stress, *ex vivo* endothelium-dependent relaxation was significantly enhanced by all four glycomimetics. Furthermore, the glycomimetics did not induce HUVEC activation, as determined by lack of ICAM-1 protein.

Conclusion We have developed a new set of small molecule glycomimetics that do not activate ECs and protect against free fatty acid-induced endothelial dysfunction both *in vitro* and *ex vivo*. Future work will focus on developing the glycomimetics into drug-like therapies that target endothelial damage.

205 INFLUENCE OF NOX NADPH OXIDASES ON HUMAN PARTIAL INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS

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Background Human induced pluripotent stem (iPS) cell-derived endothelial cells (ECs) hold clear potential for therapeutic angiogenesis as a novel strategy for ischaemic disease. Recently, our group has developed a novel method for direct reprogramming of partial iPS (PiPS) cells, which unlike iPS cells, are generated before pluripotency so do not form tumours. Importantly, PiPS cells may be differentiated into ECs with characteristic morphology and pro-angiogenic actions, which *in vitro* and *in vivo* studies have demonstrated are comparable to mature ECs with regard to their capability of forming vascular-like tubes and re-endothelialisation of ischaemic tissue. It is well established that oxidative stress and reactive oxygen species (ROS), which are characteristic features of ischaemic disease, are important regulators of both endothelial and stem cell biology, with recent evidence suggesting a key role for NADPH oxidases. Notably, we have previously identified a key role for the Nox4 isoform in regulating