terising changes in local gene expression in endothelium sampled from control coronary arteries and from advanced coronary atherosclerotic plaques.

Methods and Results A protocol was optimised to perform laser capture microdissection for isolation of endothelium in under 20 minutes, from snap-frozen porcine coronary artery cross-sections for total RNA sequencing (n=5 D374Y-PCSK9 hyperlipidaemic minipigs fed on a high fat high cholesterol diet; 10 vessels). Endothelium was sampled from control left anterior descending arteries consisting of no plaque, and from overlying advanced atherosclerotic plaque in stenotic right coronary arteries. Differential gene expression and gene ontology enrichment analyses revealed the upregulation of numerous atheroprone inflammatory genes in diseased endothelium overlying atherosclerotic plaque compared to healthy endothelium (figure 1: $p < 0.01$, $FDR < 0.05$, fold change > 2). Biological pathways related to atherosclerosis that the differentially expressed genes are most enriched in are shown (table 1: enrichment score > 3).

Conclusions We report in vivo changes in gene expression in diseased endothelium overlying advanced coronary atherosclerotic plaques, with upregulation of inflammatory genes. The differentially expressed genes are enriched in processes related to atherosclerosis, suggesting the validity of this approach to study how gene expression changes during coronary atherosclerotic plaque development in vivo. Our model system allows for further studies coupling together these readouts to a fully validated 3D vessel reconstruction method to co-register gene expression profiles to local blood flow data, as a novel methodology for understanding the mechanisms by which local flow disturbances may affect atherogenesis. This will provide new insights into how disturbed flow and coronary atherosclerotic plaque development are causally related.

Conflict of Interest None

BS19

PHOSPHODIESTERASE 1 (PDE1) INHIBITION ENHANCES PROSTACYCLIN MEDIATED SIGNALLING IN PULMONARY ARTERY SMOOTH MUSCLE CELLS THROUGH A NOVEL SELECTIVE PDE1C:PROSTACYCLIN RECEPTOR INTERACTION

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Introduction Pulmonary arterial hypertension (PAH) is associated with increased phosphodiesterase 1C (PDE1C) expression and activity, which accounts in part, to lower cAMP accumulation and increased proliferation of pulmonary artery smooth muscle cells (PASMC) isolated from PAH-patients: PDE1C expression correlates with increased PASMC proliferation. PAH-PASMC also have decreased prostacyclin (IP) receptor expression that limits their response to IP agonists. Recently, a phosphodiesterase 1 inhibitor (16K) has been developed for central nervous system disorders and tested in preclinical studies. Using PASMC we aimed to investigate the response of 16K, alone and together with the prostacyclin (IP) receptor agonist selexipag, to provide evidence for the therapeutic utility of PDE1 inhibitors for PAH.

Methods Human PASMCs were cultured under normoxia and hypoxia (1% O₂, 72 hr) to investigate PDE1C expression (Real-time PCR) and the effect of 16K (0.01-10 μ M) and

Selexipag (0.001-1 μ M) on PASMCs proliferation (MTS) and cAMP accumulation (ELISA). PDE1C, PDE1A and PDE4B cDNA (SinoBiological) and IP cDNA (cDNA.org) were used to be stably overexpressed in HEK293 (Lipofectamine 2000, ThermoFisher). Lysosomal inhibitor (chloroquine, 100 μ M) and proteasomal inhibition (MG132, 10 μ M) were used to assess IP receptor degradation, via fluorescence microscopy. Q5 sit-directed mutagenesis kit was used for prostacyclin receptor PDZ domain manipulation. Experiments were performed at least three times and data presented as means $\pm$ S.E.M and compared by ANOVA or student t-test.

Results PDE1C mRNA is increased in PASMC exposed to hypoxia (62.9 ± 6.9 -fold increase vs. normoxia, $p < 0.05$, $n=3$), which correlated with increased PDE1C protein expression and activity: increased PDE1C expression correlated with lower cAMP (103.4 ± 4.9 vs. 56.3 ± 3.5 pmol/million cells in normoxic and hypoxic PASMC, respectively) and increased proliferation. PDE1 inhibition (16K) restored cAMP levels (56.3 ± 3.5 vs. 106.2 ± 8.8 pmol/million cells in control and 16K-treated hypoxic PASMC, respectively) and induced PASMC relaxation and inhibited hypoxia-induced proliferation (21 ± 0.03 % decrease, MTS assay). Selexipag-mediated cAMP accumulation and relaxation, which was blunted in hypoxic-PASMC, was restored by 16K pre-treatment. In PASMC and HEK293 we found overexpression of PDE1C correlated with increased proteasomal degradation of the IP receptor, which blunted receptor mediated responses. Overexpression of other PDEs (PDE1A or 4B) were not associated with changes in IP receptor function. Manipulating PDZ domain of IP receptor, which binds PDZK1, prevented this interaction and restored the function and expression of IP receptor. 16K restored the expression of the IP receptor and its agonist induced cAMP accumulation via a cAMP-PKA-dependent mechanism.

Conclusions Our data show 16K increases basal and selexipag-mediated cAMP accumulation, relaxation and inhibition of proliferation in hypoxic-PASMC. We uncovered a novel PDZ-facilitated interaction of PDE1C with the IP receptor, such that increased PDE1C associated with PAH would limit agonist-induced cAMP accumulation and relaxation by enhancing IP degradation. Together these data provide further evidence that PDE1 selective inhibitors could represent a novel PAH treatment alone and importantly enhance the response to prostacyclin agonists.

Conflict of Interest None

BS20

THE OPPOSING EFFECTS OF CHRONIC INTERLEUKIN-1 β ON TIE2:TIE1 RATIO AND ANGIOPOIETIN1 INDUCED PI3 KINASE/AKT SIGNALING IN ENDOTHELIAL CELLS

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Introduction Angiotensin-1 (Ang-1) is a growth factor that plays a crucial role in maintaining normal vascular function. The main role of Ang-1 is to maintain endothelial survival. Ang-1 exerts its protective effect by activating Tie-2 receptors and subsequently the Phosphatidylinositol 3-kinase (PI3Kinase)/AKT pathway. Tie-1 regulates Ang-1 signalling with high levels of the receptor reducing Ang-1-induced Tie-2 activation. Proinflammatory cytokines including Tumor Necrotic factor (TNF- α) Interleukin 1 β (IL-1 β) have been implicated in a

range of vascular pathologies including vascular inflammation and atherosclerosis. While TNF- α has shown to regulate Tie receptors, the chronic impact IL-1 β has on Ang-1/Tie receptor signalling pathway and vascular function has not been investigated.

Aim To examine the impact IL-1 β has on Tie receptor expression and Angiopoietin-1 induced PI3Kinase/AKT activity in endothelial cells.

Method Primary Human Umbilical Vein Endothelial Cells (HUVEC) were stimulated with 25ng/ml of IL-1 β in the presence or absence of 100ng/ml of human recombinant Ang-1. The treatment times ranged from 0 to 48h. Cell lysates from the treated cells were then subjected to Western blotting to analyze Tie receptor levels and phospho-AKT (pAKT), a signaling molecule associated with Ang-1 cellular transduction. The levels of target proteins were compared between reactions by quantifying mean intensity of bands.

In addition, cells were treated with Ang-1 in the absence or presence of IL-1 β at various time points. The cell viability assay was performed on the treated cells by following the manufactures protocol. The mean percentage of live to dead cells was calculated from three random fields for each treatment.

Data for the Tie receptor and AKT analysis is presented as means and SEM of three independent experiments. Statistical significance represented with $p < 0.05$ using Student's t-test. Data for the cell viability assay is presented as means and SEM of two independent experiments.

Results A significant reduction in the levels of Tie-1 receptor was observed at 3h ($58.6 \pm 8.6\%$) in HUVECs treated with IL-1 β . The cytokine was able to maintain significant low levels of Tie1 up until 48h of treatment, whereas the changes in levels of Tie-2 were insignificant. Interestingly, IL-1 β significantly reduced Ang1-induced pAKT activity from 3h onwards with maximum reduction of $73.4 \pm 12.8\%$ observed at 48h. The cell viability assays showed reduction in the percentage live to dead cells between Ang-1 and Ang1 + IL-1 β for chronic time points tested.

Conclusion Long term exposure of Interleukin-1 β is capable of altering Tie-1 levels and increasing the Tie-2: Tie-1 ratio in endothelial cells. In contracts, IL-1 β reduces Ang-1-PI3Kinase/AKT signalling and endothelium cell viability. This opposing phenomenon observed where IL-1 β impairs Ang-1 protective ability suggests and different mechanism of regulation, independent of the Tie-1 receptor.

Conflict of Interest None

BS21

STABILISATION OF SUPPRESSOR OF CYTOKINE SIGNALLING 3 (SOCS3) TO INHIBIT HUMAN SAPHENOUS VEIN SMOOTH MUSCLE CELL PROLIFERATION AND VASCULAR STENOSIS

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Introduction Suppressor of cytokine signalling 3 (SOCS3) limits multiple signalling pathways involved in vascular inflammation and remodelling responsible for neointimal hyperplasia and vein graft failure. However, SOCS3 function is limited by its short biological half-life, suggesting that SOCS3 stabilisation might prove an effective therapeutic strategy. Through

identification of SOCS3 ubiquitination sites, we have engineered a novel SOCS3 transgene resistant to proteasomal degradation and assessed its ability to limit signalling pathways and processes responsible for neointimal hyperplasia.

Methods Flag-tagged SOCS3 transiently expressed in HEK293 cells was immunoprecipitated with anti-Flag antibody and fractionated by SDS-PAGE for trypsin digestion. Tryptic peptides were analysed by liquid chromatography and tandem mass spectrometry, and ubiquitination sites identified from di-Gly-modified SOCS3 peptides. Smooth muscle cells (SMCs) and endothelial cells (ECs) from human saphenous vein (HSV) were transduced with recombinant lentiviruses (LVs) with MOIs ranging from 3.6-22.2 tfu/cell. Ubiquitination was assessed by immunoprecipitation of soluble cell lysates and immunoblotting. Half-lives of SOCS3 transgenes were determined by immunoblotting following HSVSMC incubation $\pm$ protein synthesis inhibitor emetine. HSVSMC proliferation was assessed by cell counting. Finally, effects of WT and Lys-less SOCS3 gene delivery on cell signalling was determined by assessing phosphorylation of STAT3 (Tyr705) and ERK1/2 (Thr202/Tyr204) by immunoblotting.

Results Analysis of recombinant SOCS3 immunoprecipitated from HEK293 cells revealed that 8 of the 9 Lys residues in human SOCS3 were ubiquitinated. A mutated SOCS3 in which all 9 Lys residues were mutated to Arg was resistant to ubiquitination compared to wild type (WT) SOCS3. LV transduction of WT and Lys-less SOCS3 in HSVSMCs and ECs was highly efficient with $>90\%$ of cells expressing SOCS3 transgene after 48 hours. Lys-less SOCS3 was significantly more stable, displaying a biological half-life >4 hours versus <4 hours for WT ($n=6$, $p < 0.001$). Despite these differences, WT and Lys-less SOCS3 were functionally equivalent in their ability to selectively inhibit STAT3 phosphorylation in response to either sIL-6R α /IL-6 ($74 \pm 6\%$ and $80 \pm 7\%$ inhibition respectively; $n=4$, $p < 0.05$ versus controls) or PDGF-BB ($67 \pm 17\%$ and $72 \pm 18\%$ inhibition respectively; $n=5$) but not ERK1/2 phosphorylation. However, while WT and Lys-less SOCS3 each inhibited cell proliferation in response to sIL-6R α /IL-6 ($83 \pm 29\%$ and $89 \pm 22\%$ inhibition respectively; $n=4$, $p < 0.05$ versus controls) only Lys-less SOCS3 significantly inhibited PDGF-BB-stimulated proliferation ($67 \pm 11\%$ inhibition; $n=4$, $p < 0.05$ versus controls).

Conclusions These results provide evidence for possible therapeutic targeting of SOCS3 stabilisation to limit HSVSMC dysfunction responsible for vein graft failure.

Conflict of Interest None

BS22

TRPV4 IS A PHOSPHOLIPASE C COUPLED RECEPTOR WHEN MITOCHONDRIA ARE DEPOLARISED IN INTACT ENDOTHELIAL CELLS

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Mitochondria are significant regulators of intracellular Ca²⁺ signalling in the vascular endothelium. Depolarization of the mitochondrial membrane potential ($\Delta\psi_m$) inhibits IP₃-mediated Ca²⁺ release and may regulate Ca²⁺ influx across the plasma membrane. However, precisely how Ca²⁺ influx is regulated by mitochondria in the endothelium is unknown. To examine mitochondrial regulation of Ca²⁺ influx, the interaction of TRPV4-mediated Ca²⁺ influx with