vasculature than healthy placental sections. Thrombi could be observed in both large conduit and small resistance vessels, with many small vessels completely occluded. In FGR, total fibrin deposition in placental tissue was significantly increased ($p<0.001$), as was fibrin expression specifically located within fetal vessels ($p<0.05$). In contrast, tissue factor levels remained unaltered. Blood samples from fetal growth restricted babies demonstrated increased closure time in platelet function assays (122 ± 12.6 s) compared to healthy controls (90.10 ± 5.46 s), and platelet counts were significantly reduced ($p<0.05$). The red blood cell count, white blood cell count and mean platelet volume were not significantly different.

Conclusions These data indicate that in FGR there is increased platelet activation and thrombosis in fetoplacental vessels, which may contribute to increased vascular resistance through the occlusion of small resistance arteries. Anti-thrombotic therapies, which cross the placenta may therefore be beneficial in FGR to prevent placental thrombosis and improve pregnancy outcome.

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HAEM AND CARBON MONOXIDE (CO) MODULATION OF LARGE-CONDUCTANCE Ca^{2+} -ACTIVATED K^{+} (BK) CHANNEL ACTIVITY

Modupe Ayeni*, Sofia Kapetanaki, Emma Raven, Nina Storey, Noel Davies. *University of Leicester*

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Haemolysis is a distinctive feature of certain diseases such as haemorrhagic stroke and sickle cell anaemia. The haem released from red blood cells during haemolysis can build up to toxic levels producing further complications such as cell and tissue damage. Intracellular haem concentration is mainly regulated by haem oxygenase (HO) enzymes which degrade haem to carbon monoxide (CO) and other molecules. Nevertheless, the high haem concentrations during haemolytic diseases can saturate the HO system potentially increasing concentrations of both CO and free haem.

Arterial smooth muscle cells (SMCs) express a variety of ion channels including large-conductance Ca^{2+} -activated K^{+} (BK) channels. Activation of BK channels produce spontaneous transient outward currents (STOCs) which hyperpolarize SMCs resulting in cell relaxation and vasodilation. Therefore, BK channels have a negative feedback role to limit SMC contraction. Intracellular haem has been reported to inhibit single BK channel activity whereas CO has been shown to produce stimulatory effects. However, little is known about the effects of haem and CO on STOCs or whole-cell BK currents. The aim of this study is to investigate the effects of haem and CO on STOCs.

SMCs were isolated from the mesenteric artery of male Wistar rats. Single BK channel currents and STOCs were recorded using excised (inside-out) and perforated whole-cell patch techniques respectively. CO was applied using CO-releasing molecule 3 (CORM-3) which was prepared before each use. Data are presented as mean $\pm$ SEM and statistical analyses were performed using paired and unpaired Students T-test as appropriate. Consistent with previous reports, application of haem (100 nM) to the cytoplasmic side of inside-out patches reduced BK channel activity to $4.7 \times 10^{-3} \pm 3.4 \times 10^{-3}$ times that of control ($n=4$, $p<0.05$) whereas CORM-3 (30 μM) increased channel activity by 5.7 ± 0.4 fold ($n=8$,

$p<0.05$) at low $[\text{Ca}^{2+}]_i$ (300 nM). Surprisingly, during perforated whole-cell recordings, extracellular haem (5 μM) increased STOC amplitude by 16% (1.16 ± 0.04 , $p<0.05$, $n=3$). This effect was further enhanced in the presence of the HO-inhibitor, zinc protoporphyrin-IX (ZnPP-IX) (1.4 ± 0.1 , $p=0.01$, $n=4$). Extracellular application of CORM-3 (30 μM) increased STOC frequency (1.81 ± 0.26 , $p<0.01$, $n=8$) without significantly affecting STOC amplitude.

In summary, the stimulatory effects of haem on STOCs contradict excised patch data. This suggests that the effects of haem might be influenced by intracellular components which are absent in excised patches. Results from ZnPP-IX studies further imply that the effects of haem on STOCs might be mediated independent of its degradation product, CO. Therefore, information gained from further studies could improve our understanding of the vascular changes that occur during haemolytic diseases.

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ROLE OF MIR-214 IN ANGIOTENSIN II INDUCED HYPERTENSIVE HEART DISEASE

¹Eilidh McGinnigle*, ²Ryszard Nosalski, ³Dominik Skiba, ⁴Laura Denby, ³Delyth Graham, ⁴Andrew H Baker, ³Tomasz J Guzik. ¹*Institute of Cardiovascular and Medical Sciences, University of Glasgow*; ²*Jagiellonian University School of Medicine*; ³*Institute of Cardiovascular and Medical Sciences, University of Glasgow*; ⁴*Queens Medical Research Institute, University of Edinburgh*

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Background Cardiac dysfunction is one of the hallmarks of hypertension. It is characterised by cardiac fibrosis, diastolic dysfunction and development of heart failure. The mechanisms of hypertensive heart disease are not fully understood. Recent studies suggest a role of miR-214 in regulation of fibrosis. Thus, the aim of this work is to investigate the role miR-214 has in the cardiac sequelae of hypertension.

Methods Hypertension was induced in three month old miR-214 knock out (KO) and wild type (WT) littermates using 490 ng/kg/min angiotensin II (Ang II) for 14 or 28 days ($n=4-6$). Blood pressure was monitored by tail cuff plethysmography. Cardiac fibrosis was assessed by picrosirius red and Massons trichrome staining. Mir214 expression was assessed by Taqman and *in situ* hybridisation (ISH). Cardiac function was assessed by echocardiography. Effects of Ang II on fibrosis genes was studied in primary cardiac fibroblasts.

Results 14 day Ang II infusion caused 4-fold induction of miR-214 in the left ventricle of C57bl/6 mice ($p<0.001$) which was confirmed by ISH. Loss of miR-214 was associated with increased cardiac fibrosis in picrosirius red ($2.8\% \pm 0.52$ vs. $4.8\% \pm 0.7$; $p<0.005$) caused by Ang II, without effect on blood pressure. This was associated in a significant up-regulation of *COL1A1* mRNA compared with their WT littermates ($p<0.01$). Echocardiography revealed enhanced development of ventricular wall thickening and diastolic dysfunction in mir214 KO when compared to WT mice (E/A ratio: 1.79 ± 0.39 vs 0.59 ± 0.04 ; $p<0.05$). In contrast to *in vivo* observations, *in vitro* fibroblast studies show that KO fibroblasts showed significantly reduced induction of *COL1A1* ($p<0.05$) and *COL3A1* ($p<0.05$) compared with WT fibroblasts upon Ang II and IL-17 suggesting that involvement of miR-214 in cardiac dysfunction is not directly modulated by the effects in fibroblasts but through other mechanisms that need to be further addressed.

Conclusions Cardiac fibrosis, hypertrophy and diastolic dysfunction are enhanced in miR-214 KO animals. The mechanisms of this observation are not caused by mir214 effects in cardiac fibroblasts, but through other mechanisms that need to be further addressed.

192 THE EFFECT OF INTERLEUKIN 1 ON VASCULAR ANGIOPOIETIN 1 SIGNALLING

Jay Bilimoria*, Harprit Singh. *De Montfort University*

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Introduction Angiopoietin 1 (Ang1) is a growth factor that plays a crucial role in maintaining normal vascular function. The main role of Ang1 is to maintain vessels in quiescent, inhibit vascular inflammation and maintain endothelial survival. Ang1 exerts its protective effect by activating Tie2 receptors, which are predominantly expressed on endothelial cells. The ratio between Tie2 and its co-receptor, Tie1, is a regulatory factor in Ang1 signalling with high levels of Tie1 capable of reducing Ang1-induced Tie2 activation. The activity of Ang1 is aided by Tie2 phosphorylation followed by activation of cascade of downstream signalling pathways including Phosphatidylinositol 3-kinase (PI3Kinase)/AKT and Erk1/2. Interleukin 1 Beta (IL1 β) is a proinflammatory cytokine that acts on microvasculature and large vessels and has been implicated in a range of vascular pathologies including vascular inflammation and atherosclerosis. Various pathophysiological mediators including VEGF and TNF alpha have shown to affect the levels of Tie receptor expression and subsequently influencing Ang1 signalling in endothelial cells; however the involvement of Interleukin 1 β on this pathway has not been reported.

Aim To examine the impact IL1 β has on Tie2:Tie1 expression ratio and Angiopoietin1 signalling in endothelial cells.

Method Primary Human umbilical vein endothelial cells (HUVEC) were stimulated with 25 ng/ml of IL1 β at different time periods in the presence or absence of 300 ng/ml of human recombinant Ang1. Cell lysates from the treated cells were then subjected to immunoprecipitation and Western blotting to analyse Tie receptor levels and signalling molecules associated with Ang1 cellular transduction including Phospho-Tie2 (pTie2), and phospho-AKT (pAKT). The levels of target proteins were compared between reactions by quantifying mean intensity of bands. Data is presented as means and SEM of three independent experiments. Statistical significance represented with $p < 0.05$ using Students t test.

Results The expression of Tie2 and Tie1 protein in endothelial cells treated with IL1 β was significantly altered with different Tie2:Tie1 ratio patterns observed at 1 hour and 24 hour. These changes to the Tie2:Tie1 expression patterns also altered the ability of Ang1 to induce Tie2 phosphorylation and downstream signalling molecules including pAKT.

Conclusion This study demonstrates for the first time that IL1 β is capable of altering Tie2:Tie1 ratio that subsequently leads to the regulation of Ang1 signalling. These findings provide further insight on how the Angiopoietin1 signalling pathway adapts to the acute and chronic effect of IL1 β and how this signalling pathway is important in limiting pro-inflammatory responses of the vasculature.

193 THE ROLE OF THE PI3K-ALPHA ISOFORM IN CARDIOPROTECTION

Xavier Rossello*, David He, Jaime A Riquelme, Sean Davidson, Derek M Yellon. *The Hatter Cardiovascular Institute, University College London*

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Introduction Ischaemic preconditioning (IPC) limits infarct size (IS) through the activation of the PI3K-AKT signal cascade, known as the RISK pathway. The identification of the roles of individual PI3K isoforms should aid in the development of isoform-specific agents with improved cardioprotection. We aimed to elucidate the role of the PI3K α isoform in cardioprotection.

Methods Male C57BL/6 mice were randomly allocated between groups in isolated perfused Langendorff experiments subjected to 35 min ischaemia and 2 hour reperfusion. Two distinct PI3K α -isoform specific inhibitors (3 μ M of G326 and BYL719) were administered during the IPC protocol (4-cycles of 5 min ischaemia and 5 min reperfusion), or at reperfusion. PI3K activity was measured by Western blot analyses of AKT phosphorylation. Insulin was used as PI3K α canonical activator. In addition, these studies were also undertaken using an *in vivo* mouse model. Finally, expression of PI3K α protein levels was evaluated by Western blot in whole mouse heart and human atrial tissues (REC No. 00/0275), as well as in primary adult ventricular mouse cardiomyocytes and mouse cardiac endothelial cell (MCEC) line.

Results In the isolated Langendorff-perfused model, IPC reduced IS compared to control ($49 \pm 4\%$ vs $23 \pm 2\%$, $p < 0.001$). Protection was not abolished using either G326 ($26 \pm 3\%$) or BYL719 ($25 \pm 3\%$) when administered during IPC (Figure 1A) despite the phosphorylation of AKT being inhibited. Interestingly, these same isoform inhibitors did block protection when given at reperfusion (G326 $50 \pm 3\%$; BYL719 $47 \pm 4\%$) (Figure 1B), in addition to blocking the AKT phosphorylation revealing a distinct role for the PI3K α isoform during the early moments of reperfusion. Neither drug affected IS on its own. In support, the canonical PI3K α activator, insulin, when given at reperfusion reduced myocardial IS compared to control (25 ± 2 vs $55 \pm 4\%$, $p < 0.001$) and this protection was abolished by G326 (Figure 2A). These experiments were confirmed *in vivo* when using the PI3K α isoform inhibitor at reperfusion to block IPC protection (Figure 2B). On a tissue level, PI3K α is expressed at the same level in mouse heart and human atrium. Interestingly, PI3K α levels in MCEC were about 3-fold higher compared to adult ventricular mouse cardiomyocytes ($p = 0.047$).

Conclusions Using a targeted pharmacological approach, we have demonstrated that the PI3K α isoform is required during the reperfusion phase for protection conferred by IPC. The translational perspective is highlighted by the observation that human heart tissue expresses PI3K α . This suggests that strategies specifically enhancing the α isoform of PI3 kinase at reperfusion promotes tissue salvage and, as such, could provide an attractive target for clinical treatment of ischaemia-reperfusion injury.