± 0.28 ischaemic/control limb ratio; $n=8$, $p<0.05$) CB-ECFCs into mouse ischaemic hindlimbs inhibited and promoted revascularisation whilst regulating host eNOS-associated angiogenic signalling. Together, these findings indicate a key role for NOX4 in CB-ECFCs, highlighting its potential as a target for enhancing their reparative function through therapeutic priming to support creation of a pro-reparative microenvironment and promotion of effective post ischaemic revascularisation.

9 INVESTIGATING THE COUNTER REGULATORY RENIN ANGIOTENSIN SYSTEM AXIS IN THE STROKE PRONE SPONTANEOUSLY HYPERTENSIVE RAT IN ISCHAEMIC STROKE

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Several studies have assessed the potential of targeting the renin angiotensin system (RAS) with therapeutics for ischaemic stroke. The counter regulatory RAS peptide, angiotensin-(1-9) has been shown to act via the angiotensin II type 2 receptor (AT₂R) to oppose detrimental effects of RAS dysregulation. We hypothesise that Ang-(1-9) may have a beneficial effect on stroke outcome in the spontaneously hypertensive stroke prone rat (SHRSP). Initial qPCR experiments have assessed temporal changes in RAS gene expression (angiotensin converting enzyme 2; ACE2, AT₂R; AGTR2, Mas receptor; Mas) following 35 min transient middle cerebral artery occlusion (tMCAO) followed by varying reperfusion times: no reperfusion ($n=4$), 2 hour ($n=4$) and 24 hour ($n=4$), compared to sham surgery ($n=7$).

In infarcted tissue, there was a significant 10- and 11-fold reduction in ACE2 and Mas expression respectively, 24 hour post tMCAO vs sham (RQ+RQ_{max}: ACE2: sham 1.0+0.2; 24 hour post tMCAO 0.1+0.01, $p<0.01$, Mas: sham 1.0+0.2; 24 hour post tMCAO 0.09+0.03 $p<0.01$). However, in the same tissue, AGTR2 showed a 4-fold increase in expression after 35 min occlusion vs sham (RQ+RQ_{max}: sham 1.0+0.3; 35 min MCAO 4.2+0.2, $p<0.05$). Additionally, in the sub-cortical remainder tissue, ACE2 and AGTR2 expression decreased by 2.5- and 5-fold respectively 24 hour post tMCAO (RQ+RQ_{max}: ACE2: sham 1.0+0.1; 24 hour post tMCAO 0.4+0.1, $p<0.05$, AGTR2: sham 1.0+0.4 ; 24 hour post tMCAO 0.2+0.1 $p<0.05$).

These results demonstrate altered counter regulatory RAS gene expression in the ipsilateral hemisphere in the 24 hours following tMCAO in SHRSP. Additional experiments have demonstrated successful transduction of a control reporter gene-expressing, adeno-associated virus serotype 9 (AAV9) expressing enhanced green fluorescent protein (eGFP) (AAV9-eGFP) in the SHRSP brain via stereotactic delivery after both 4 and 7 days. Future studies will assess the therapeutic potential of Ang-(1-9) in tMCAO induced experimental stroke in SHRSP by delivering Ang-(1-9) via stereotactic delivery of an AAV9 vector.

10 ENDOGENOUS AND EXOGENOUS LOADING OF EXTRACELLULAR VESICLES FOR THERAPEUTIC DELIVERY OF RENIN-ANGIOTENSIN SYSTEM PEPTIDES IN CARDIOMYOCYTE HYPERTROPHY

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Introduction The RAS peptide angiotensin II (AngII) mediates cardiac hypertrophy. The counter-regulatory RAS axis peptide Angiotensin 1-7 [Ang-(1-7)] inhibits cardiomyocyte hypertrophy. EVs were purified from cardiomyocytes $\pm$ treatment with AngII or Ang-(1-7) to assess cardiomyocyte hypertrophy. EVs were loaded with Ang-(1-7) via electroporation for therapeutic delivery.

Methods H9c2 cardiomyocytes were untreated (control) or treated with AngII or Ang-(1-7). EVs were isolated from conditioned media by differential ultracentrifugation, characterised by BCA, western immunoblot, Nanosight and TEM and incubated with recipient cardiomyocytes. Next, cells were stained with F-Phalloidin actin and area measured. Gene expression of hypertrophy marker brain natriuretic peptide (BNP) was assessed by qRT-PCR. Control EVs were electroporated in the presence of Ang-(1-7) and levels determined by ELISA.

Results H9c2 cardiomyocyte-derived EV size was 101.0 ± 2.4 nm and EV markers CD63 and TSG-101 were consistently detected. EVs from AngII treated cardiomyocytes significantly increased recipient cardiomyocyte area compared to control EVs [control: $3291.1 \pm 90.1 \mu\text{m}^2$ vs AngII: $5252.3 \pm 125.4 \mu\text{m}^2$; $p<0.001$] and significantly increased BNP expression [$p<0.017$]. EVs isolated from Ang-(1-7) treated H9c2 cardiomyocytes significantly reduced AngII induced hypertrophy in recipient cardiomyocytes [AngII +Control EVs: $5566.3 \pm 139.0 \mu\text{m}^2$ vs AngII +Ang-(1-7) EVs: $4212.7 \pm 132.1 \mu\text{m}^2$; $p<0.01$]. Electroporation loaded EVs with Ang-(1-7) [naïve EVs: 0.0 pg/mL vs Ang-(1-7) EVs: $342.3 \pm 9.1 \text{ pg/mL}$; $p<0.001$]. Ang-(1-7) loaded EVs significantly reduced AngII induced hypertrophy in recipient cardiomyocytes [Naïve EVs: $4641.2 \pm 35.3 \mu\text{m}^2$ vs Ang-(1-7) EVs: $2758.4 \pm 20.1 \mu\text{m}^2$; $p<0.001$].

Conclusion EVs isolated from AngII treated H9c2 cardiomyocytes stimulate recipient cardiomyocyte hypertrophy. EVs isolated from Ang-(1-7) treated cardiomyocytes inhibit hypertrophy. Furthermore, EVs exogenously loaded with Ang-(1-7) inhibit cardiomyocyte hypertrophy. These findings have implications for understanding the role of the RAS and EV function in cardiomyocytes.

Poster Presentations

1 A SURVEY EVALUATING HEALTHCARE PROFESSIONALS' KNOWLEDGE AND PERCEPTIONS OF ELECTRONIC CIGARETTES

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