±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

[1]Aisling McFall, [2]Lorena Zentilin, [2]Mauro Giacca, [1]Stuart A Nicklin, [1]Lorraine M Work. [1]*Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK*; [2]*International Centre for Genetic Engineering and Biotechnology, Trieste, Italy*

10.1136/heartjnl-2018-SCF.9

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 (AT_2R) 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*, AT_2R; *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 subcortical 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

LS Downie, LM Work, SA Nicklin. *Institute of Cardiovascular and Medical Sciences, University of Glasgow*

10.1136/heartjnl-2018-SCF.10

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 ± 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±90.1 μm^2 vs AngII:5252.3 ±125.4 μm^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±139.0μm^2 vs AngII +Ang-(1–7) EVs:4212.7 ±132.1 μm^2; p<0.01]. Electroporation loaded EVs with Ang-(1–7) [naïve EVs:0.0 pg/mL vs Ang-(1–7) EVs:342.3±9.1 pg/mL; p<0.001]. Ang-(1–7) loaded EVs significantly reduced AngII induced hypertrophy in recipient cardiomyocytes [Naive EVs:4641.2±35.3μm^2 vs Ang-(1–7) EVs:2758.4±20.1μm^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

Dickson Dewantoro, Danièle MI Kerr, Christian Delles. *Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK*

10.1136/heartjnl-2018-SCF.11