±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*

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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 (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*

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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 ± 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*

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Introduction Electronic cigarettes (EC) are currently the preferred nicotine replacement product to support tobacco smoking quit attempts. Despite the potential harm reduction associated with EC, their use remains controversial. The aim of this study was to evaluate doctors' knowledge and perceptions of NRT and EC.

Methods An online and paper survey was distributed to healthcare professionals working within NHS Greater Glasgow and Clyde from 3rd to 19th October 2017.

Results 2291 healthcare professionals completed the survey of which 338 were completed by doctors and were included in this analysis. Out of these, 83.2% (n=281) regularly see patients who smoke tobacco cigarettes. When asked the question 'Do you think EC are a good thing?', 30.2% (n=102) disagreed; 31.3% (n=106) agreed and 38.5% (n=130) remained neutral. The majority of doctors perceived that nicotine replacement patches (NRP) and EC were less harmful in comparison to tobacco smoking (NRP 97.3%, n=329; EC 83.4%, n=282). 53.3% (n=180) of doctors said they would recommend EC as a method to stop smoking, while 46.7% (n=158) would not. 65.5% (n=222) of doctors agreed that they did not feel confident about advising patients regarding EC use and 76.1% (n=257) felt that they required more information and guidance.

Conclusions Whilst the majority of doctors perceived EC as a safer alternative to tobacco smoking there is a discrepancy between their perceptions and what they would clinically recommend to patients. Our data highlight that in the context of smoking cessation and the unknown long-term health effects from EC exposure, doctors may benefit from having access to medical evidence and latest recommendations regarding EC.

2 CHOLESTEROL CRYSTAL SECRETION OF IL-1β FROM PBMCS IS REDUCED WITH SIMVASTATIN TREATMENT

[1]N Gangadharan, [1]P Kavanagh, [2]PT Wash, [1]L Hemeryck, [1]J Kieran, [1]M Barry, [1]M Lucitt. [1]*Pharmacology and Therapeutics, Trinity College Dublin, Dublin, Ireland*; [2]*Clinical Medicine, Trinity College Dublin, Dublin, Ireland*

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Considerable evidence implicates a role for interleukin-1 beta (IL-1β) in the pathogenesis of atherosclerosis[1] revealing its potential as a novel therapeutic target. Statins are known to have anti-inflammatory effects,[2] however the specific mechanisms remain to be established. To test the anti-inflammatory effects of simvastatin, PBMCs were isolated from healthy donors and treated *in vitro* with simvastatin (100 μM) or from hyperlipidaemic patients at baseline and following 8 weeks simvastatin (10–20 mg) daily treatment. PBMCs were then stimulated with LPS (100 ng/ml) for 3 hour followed by cholesterol crystal (CC) (1 mg/ml) stimulation overnight to activate the NLRP3 inflammasome complex involved in processing IL-1β to its mature secreted form. IL-Iβ levels in the supernatants form PBMCs was measured by ELISA. All experiments carried out were approved by the Medical Research Ethics Committees at St James Hospital/AMNCH, Dublin 8, Ireland and comply fully with the Declaration of Helsinki. Patients (n=9) taking simvastatin (10–20 mg daily) over 8 weeks exhibited reduced LDL cholesterol, (4.87±0.76 mmol/L) pre vs (3.78±0.67 mmol/L) post statin treatment. Simvastatin treatment also reduced levels of IL-1β secretion by PBMCs, when stimulated with LPS and CC, (5.27±0.6 ng/ml) pre vs (4.27±0.5 ng/ml) post statin treatment. Similarly, *in vitro* treatment of PBMCs with simvastatin (100 μM) reduced IL-1β secretion upon activation with LPS and CC, (2.37±0.17 ng/ml) control vs (0.64±0.06 ng/ml) simvastatin treatment. Values presented are mean ±sem. We have demonstrated that CC induced IL-1β release by PBMCs from hyperlipidaemic patients, is reduced after treatment with simvastatin. These data identify a previously unappreciated beneficial role for statin therapy in atherosclerotic patients.

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3 RED YEAST RICE EXTRACT REDUCES IL-1β SECRETION FROM PBMCS WHEN TREATED WITH CHOLESTEROL CRYSTALS

[1]PV Kavanagh, [2]PT Walsh, [1]M Lucitt. [1]*Pharmacology and Therapeutics, Trinity College Dublin, Dublin, Ireland*; [2]*Clinical Medicine, Trinity College Dublin, Dublin, Ireland*

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Red yeast rice (RYR) nutraceutical contains monacolin K, a natural occurring statin.[1] Here we compare lovastatin and simvastatin effects on interleukin-1 beta (IL-1β) levels to that of extracts from a RYR product. Standard curves using 0.375–24 μg/mL of lovastatin pre and post hydrolysis with NaOH were constructed using liquid chromatography-mass spectrometry (LC-MS), operated with selected ion monitoring (SIM) and a m/z of 427 to detect the lactone and m/z of 445 to detect the hydroxyl acid forms of lovastatin. RYR, 0.22 μm filtered ethanol extractions pre and post hydrolysis were analysed by LC-MS for monacolin k lactone (inactive) and hydroxyl acid (active) forms and quantified from the standard curves generated above. Human PBMCs treated *in vitro* with lovastatin (100 μM), simvastatin (100 μM) or RYR extracts (9 μg/ml, 18 μg/ml), stimulated with LPS (100 ng/ml) for 3 hours, followed by cholesterol crystals (CC) (1 mg/ml) stimulation overnight to induce secretion of IL-Iβ, was subsequently measured by ELISA. Data shown as mean ±S.E.M, n=3. Extracts from 9 g of RYR (3 capsules) analysed by LC-MS detected 1.86–2.43 mg/capsule of the lactone. Extract levels of monacolin K lactone to monacolin K hydroxyl acid, pre and post hydrolysis was determined, (15:1) pre and (1:2) post hydrolysis. PBMCs treated with lovastatin or simvastatin reduced secreted levels of IL-1β, (0.99±0.05 ng/ml) lovastatin, (1.75±0.14 ng/ml) simvastatin vs (2.6±0.47 ng/ml) LPS and CC treatment alone. Similarly, *in vitro* treatment of PBMCs with RYR extracts also reduced IL-1β secretion, (1.15±0.09 ng/ml) RYR 18 μg/ml and (1.49±0.28 ng/ml) RYR 9 μg/ml vs (2.6±0.47 ng/ml) LPS and CC treatment alone. LPS and CC induced IL-1β release from PBMCs is reduced when treated with RYR extracts and is comparable to IL-1β release effects seen with prescription statins. These data identify a previously unappreciated anti-inflammatory effect of RYR supplements.

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