Polyphenols are regarded to have a wide range of health-promoting effects. Increased consumption of polyphenol-rich food is known to be associated with numerous cardioprotective effects. Polyphenols have been shown to improve endothelial function, inhibit abnormal platelet aggregation, reduce inflammation and improve plasma lipid profiles. Moreover, polyphenols have been widely recognised as powerful antioxidants. Given that oxidative stress plays a key role in initiation and progression of atherosclerosis, antioxidant therapy with polyphenols has potential. However, the concentrations required to mediate sufficient antioxidant effect appear to be unattainable under *in vivo* conditions. Polyphenols are characterised by poor absorption, rapid degradation and extensive metabolism, culminating in poor bioavailability (~1 μM). In addition, they can also exhibit paradoxical pro-oxidant activities.

Spectrophotometric and mass spectrometry (LC-MS/MS) analysis of the phenolic compound, delphinidin, confirmed its low stability and rapid degradation ($t^1/_2$ ~30 min) under physiologically relevant conditions. Delphinidin degraded to smaller phenolics: gallic acid and phloroglucinol aldehyde. Moreover, both the parent compound and its main metabolite, gallic acid, generated oxygen-centred free radicals at concentrations≥10 μM, as determined by electron paramagnetic resonance spectrometry (EPR). Interestingly, the tested phenolics offered significant protection to human umbilical endothelial cells (HUVECs) against chemically induced oxidative stress. The protective effect of both phenolics were hormesic in profile; supraphysiological concentrations (100 μM) were cytotoxic, whereas physiologically relevant concentrations (100 nM – 1 μM) were protective against oxidative stress. The observed protection was associated with increased intracellular glutathione.

The results confirm that physiologically relevant concentrations of delphinidin and its major metabolite, gallic acid, are sufficient to induce antioxidant benefits, but via an indirect, xenobiotic mechanism that induces upregulation of endogenous antioxidant capacity. The findings emphasis that stability, rate of absorption, distribution and metabolism of phenolic compound needs to be taken into consideration when designing *in vitro* studies to test their mechanism of action.

21 THE ROLE OF OSTEOPONTIN IN LEFT VENTRICULAR HYPERTROPHY

Tamara P Martin*, Maria Cobo Andrade, Christopher Loughrey, Delyth Graham, Martin W McBride. *Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK*

10.1136/heartjnl-2017-311433.21

The stroke-prone spontaneously hypertensive rat (SHRSP) develops increased left ventricular mass index (LVMI) prior to the onset of hypertension, making it a fundamental model to better understand human cardiovascular disease. We identified a quantitative trait locus (QTL) for LVMI on chromosome 14 and, by using chromosome 14 congenic strains and gene profiling, have identified osteopontin (*Spp1*) as a positional candidate gene. Here, we show 1) that Spp1 may promote cardiac remodelling via extracellular vesicle (EV) signalling and 2) provide phenotypic and molecular characterisation of a CRISPR/Cas9 Spp1-knockout rat on the SHRSP genetic background (SHRSP-*Spp1* KO). 1) Briefly, H9c2 cells were seeded 24 hours prior to transfection with *Spp1* cloned into pcDNA1 (5 ug) for 48 hours. EVs were isolated from conditioned media (CM) via ultracentrifugation, verified by NanoSight, re-suspended in PBS and placed onto fresh H9c2 cells for 48 hours. Crystal violet stained H9c2 cells were analysed using ImageJ. Cells transfected with *Spp1* cDNA derived from the SHRSP rat displayed a significant increase in cell size compared with cells transfected with empty pcDNA vector (pcDNA 107.9±1.4 vs SHRSP 141.8±2.3, $p<0.001$). Similarly, conditioned media (CM) taken from SHRSP transfected cells produced a significant increase in fresh H9c2 size compared with empty pcDNA vector (pcDNA 67.9±1.1 vs SHRSP 133.0 ±2.9, $p<0.001$). EVs isolated from media conditioned from cells transfected with SHRSP significantly increased fresh H9c2 cell size compared to empty pcDNA vector (pcDNA 96.6±1.5 vs SHRSP 152.9±2.6, $p<0.001$). Collectively these data suggest that over-expression of Spp1 promotes an increase in cell size via EV signalling. Further studies are required to characterise EV content and the downstream mechanisms leading to hypertrophy. 2) Hemizygous rats were bred and confirmation of *Spp1* gene knockout was confirmed in resultant pups by a restriction fragment length polymorphism, Sanger sequencing and ELISA. Echocardiography and radiotelemetry were used to assess cardiac function and blood pressure, respectively. Male rats were assessed over 5–16 weeks of age. Cardiac fibrosis was assessed by picro-sirus red staining of total collagen and Col1a1 mRNA expression was assessed by qRT-PCR. LVMI, calculated from either echocardiography or post-mortem, showed no significant difference in SHRSP-*Spp1* KO compared to its SHRSP littermate controls ($p>0.05$). Similarly, no difference was observed in LV relative wall thickness in SHRSP-*Spp1* KO compared to SHRSP ($p>0.05$). Cardiac fibrosis assessed by both histology and qRT-PCR showed no significant difference in SHRSP-*Spp1* KO compared to SHRSP ($p>0.05$). Overall, these data suggest that the cardiac phenotype of the SHRSP-*Spp1* KO rat is no different from SHRSP at baseline. Further studies are required to determine whether a cardiac stress will unmask a difference in phenotype in the SHRSP-*Spp1* KO compared to littermate controls.

22 5α-TETRAHYDROCORTISONE EXHIBITS TOPICAL ANTI-INFLAMMATORY ACTION WITH LIMITED ADVERSE EFFECTS ON ANGIOGENESIS

Amber J Abernethie*, Annalisa Gastaldello, Dawn EW Livingstone, Brian R Walker, Patrick WF Hadoke, Ruth Andrew. *Centre for Cardiovascular Science, Queens Medical Research Centre, University of Edinburgh, Scotland, UK*

10.1136/heartjnl-2017-311433.22

Glucocorticoids (GC) are potent anti-inflammatory drugs but have debilitating side effects. A safer alternative is required and 5α-tetrahydrocorticosterone (5αTHB, a metabolite of the natural rodent glucocorticoid corticosterone) may provide a solution. 5αTHB is anti-inflammatory *in vivo* but with fewer systemic adverse effects. It is rapidly cleared from systemic circulation and is therefore being investigated for topical application. Topical steroids additionally impair wound healing, largely due to the inhibition of angiogenesis and of collagen deposition. Here, the effects of 5αTHB on processes involved with wound repair were investigated.

Angiogenesis was assessed using two murine models. In the first, sponges containing vehicle, 5αTHB (3 mg or 15 mg), or corticosterone (3 mg) were implanted subcutaneously in

C57Bl6 mice (male, aged 8–12 weeks, 8–12/group). After 21 days sponges were excised and fixed, and vessel growth and collagen deposition were assessed by counting and picro-sirius red staining, respectively. At equipotent anti-inflammatory doses, corticosterone decreased the amount of collagen in sponges (3 mg changing to 30%±8% of control; $p<0.05$) whereas 5αTHB did not (15 mg; 128%±20%). Similarly, while corticosterone reduced new vessel growth to 15%±3% of control ($p<0.05$), this effect was less marked with 5αTHB (46%±7%; $p<0.05$) vs corticosterone.

In the second model, vessel growth was stimulated from mouse aortic rings (C57Bl6 mice, male, age 8–10 weeks) and attenuation of growth was assessed following incubation with steroid (dose ranges 1 nM–10 μM, n=6–8/dose). Angiogenesis was suppressed to a lesser extent by 5αTHB (EC50 2399 nM) than by dexamethasone (8 nM) or hydrocortisone (675 nM; n=6–8/dose).

These data suggest that 5αTHB could provide a safer anti-inflammatory treatment for topical application. It has fewer adverse effects on angiogenesis and collagen deposition than hydrocortisone and is thus likely to be less detrimental during wound repair.

23 NADPH OXIDASE 5 (NOX5), CHOLESTEROL-RICH MICRODOMAINS AND ANG II SIGNALLINGSIGNALING IN HUMAN PRIMARY VASCULAR SMOOTH MUSCLE CELLS

A Anagnostopoulou*, P Persson, A Montezano, R Touyz. *BHF Glasgow Cardiovascular Research Centre, Institute of Cardiovascular and Medical Sciences, University of Glasgow, 126 University Place, Glasgow, UK*

10.1136/heartjnl-2017-311433.23

Background and hypothesis Nox5 has been identified in human vessels. However the trafficking, regulation and function of vascular Nox5 is unclear. We questioned the function of Nox5 and other Nox isoforms in signalling associated with vascular smooth muscle cell contraction, growth and cytoskeletal organisation and assessed whether lipid rafts/caveolae play a role in Nox5 regulation.

Methods Human primary vascular smooth muscle cells (VSMCs) isolated from small arteries were studied. Nox isoforms were sequentially downregulated by siRNA. siRNA to p22phox reduced activity of Nox1,2 and 4, since these isoforms are p22phox-dependent. Nox5 was targeted by Nox5 siRNA. Signalling pathways associated with contraction (MLC, MYPT1), cell growth (PCNA, p53) and cytoskeletal organisation (Ezrin-Radixin-Moiesin) were assessed by immunostudies. Cells were stimulated with Ang II in the absence and presence of disrupters and enhancers of cholesterol-rich microdomains. Nox5 interaction with lipid rafts/caveolae was examined by immunohistochemistry in intact vessels and by sucrose gradient cell fractionation in cultured cells.

Results Nox5 inhibition by siRNA was associated with reduced Ang II-induced phosphorylation of MLC20 (by 0.74-fold, 5 mins stimulation) and MYPT1 (by 0.5-fold, 5 mins stimulation). p22phox siRNA did not significantly alter activation of MLC20 or MYPT1. siRNA to p22phox and Nox5 decreased Ang II-induced increase in PCNA expression (by 0.18-fold and 0.3-fold in Ang II 2 hour and 8 hour stimulation respectively, $p<0.05$) and decreased phospho-p53 levels (by 0.4-fold in Ang II 5 mins stimulation, $p<0.05$). In isolated human small arteries Nox5 co-localised with caveolin-1 as assessed by immunohistochemistry. In fractionated cells, Nox5 and Nox1 localised in fractions 3 and 4, which are rich in cholesterol microdomains. To further explore the role of lipid-rafts/caveoale in Nox function, we disrupted lipid-rafts/caveolae using methyl-b-cyclodrexin (MCD) and nystatin. MCD and nystatin increased Ang II-induced Nox-derived superoxide formation and increased Ang II-induced phosphorylation of MLC20 (by 3.45-fold and 1.12-fold respectively) and Ezrin-Radixin-Moiesin (by 1.2-fold in MCD, Ang II 15 mins stimulation and 1.2-fold in nystatin, Ang II 5 mins stimulation).

Conclusions These results indicate that in human VSMCs Ang II stimulates contractile pathways through Nox5-dependent pathways whereas signalling associated with VSMC growth and cytoskeletal organisation involve multiple Nox isoforms. Nox5 co-localises in lipid rafts/caveolae, which may play a role in Nox5 trafficking. Our data identify novel redox signalling processes through cholesterol-rich microdomains and highlight a potentially important function of Nox5 in vascular contraction.