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### **The polyphenol delphinidin induces antioxidant effects in human umbilical vein endothelial cells through activation of endogenous glutathione: importance of using relevant concentration in in vitro systems**

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the emergence and growth of collateral arteries in the thigh (upstream of the ligation point).

**Conclusion** A pre-existing collateral circulation provides the residual blood supply after femoral ligation. A rapid increase in the diameter of a small number of collateral arteries appeared to be the major mechanism for acute restoration of blood supply to the ischaemic lower leg and foot pad. Future work will use histology and immunohistochemistry to investigate the role of angiogenesis in reperfusion following femoral ligation.

**18 TOWARDS NON-INVASIVE CHARACTERISATION OF RE-ENDOTHELIALISATION AND RESTENOSIS FOLLOWING CORONARY STENTING: AN IN VITRO INVESTIGATION USING IMPEDANCE SPECTROSCOPY**

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Following the permanent implantation of a coronary stent, optimal arterial wall healing is characterised by re-endothelialisation, the regrowth of a functional Endothelial Cell (EC) monolayer over the exposed stent surface, which reduces the risk of thrombosis. However restenosis, arising from the proliferation and migration of medial Smooth Muscle Cells (SMCs) can cause luminal narrowing to reoccur. Previous research has suggested that the stent itself could be used as an electrode and, when combined with non-invasive impedance spectroscopy techniques, monitor post stenting recovery. This could then inform clinicians on cell regrowth without the need for invasive imaging techniques. In this study we investigated the feasibility of this concept using two *in-vitro* models representing the cellular regrowth scenarios: re-endothelialisation and restenosis.

Primary porcine ECs and SMCs were seeded onto platinum electrodes and electrical impedance spectroscopy measurements were made for up to 10 days in the frequency range 1 KHz to 100 KHz. Endothelium function was assessed through the measurement of the impedance response of confluent EC monolayers to the addition of a gap junction enhancer, dipyrindamole, or an inhibitor (heptanol or carbenoxolone).

Our results show that confluent, stent surface comparable populations of SMCs and ECs give rise to distinct impedance signatures, providing a novel method of non-invasively characterising these cell types. Gap junction inhibition of EC monolayers dose dependently reduced total impedance. Conversely dipyrindamole's enhancing effect on gap junction formation caused an increase in total impedance. These novel findings show the importance of intercellular gap junction communication in maintaining EC barrier function. Our current work is focused on the translation of this technology towards *in-vivo* monitoring of in-stent restenosis and recovery of a functional endothelium.

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**19 ADVERSE CARDIAC REMODELLING UNDER PRESSURE OVERLOAD. A MAGNETIC RESONANCE IMAGING STUDY IN MICE**

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Pressure overload, a hallmark of valvular heart disease and hypertension, is the leading cause of heart failure. With the progressive nature of this condition a better understanding of the process underlying the transition to heart failure is vital. Recent studies suggest that interstitial myocardial fibrosis occurs early in this transition and has a profound effect on cardiac function. The recently developed T1-mapping Cardiovascular Magnetic Resonance Imaging (CMR) technique has the potential to quantify the extracellular volume fraction (ECV) and therefore evaluate the expansion of the extracellular matrix (primarily diffuse fibrosis) over time.

We aimed to assess the feasibility of CMR (including functional and ECV imaging) to monitor cardiac remodelling using an animal model of pressure overload heart disease.

Fifteen mice were subjected to a 6 week Angiotensin-II infusion (AngII). CMR (cine and T1 mapping) was performed before and following Angiotensin II infusion at 2, 4 and 6 weeks. ECV was calculated from the T1 relaxation times pre and post-contrast infusion).

Mean blood pressure increased from 65±12 (baseline) to 84±14 mmHg (p<0.001) and ECV increased from 24.28%±3.35% (baseline) to 30.03%±5.34% after 2 weeks of AngII (p=0.011). ECV plateaued at 4 and 6 weeks and stayed significantly higher compared to baseline (p=0.001). Cine imaging revealed left ventricular (LV) hypertrophy during infusion which remained stable at 4 and 6 weeks. Interestingly, systolic function was maintained after 2 and 4 weeks of AngII but was impaired at six weeks (EF 56.3% compared to 64.4% at baseline and 59.8%; 60.7%, at 2 and 4 weeks (p=0.014)). This drop in cardiac performance was accompanied by a trend towards LV dilatation at 6 weeks compared to baseline (LV end diastolic volume 68 µl vs 63 µl, p=0.056).

Prolonged pressure overload results in ECV expansion, LV hypertrophy and subsequent systolic dysfunction. T1 mapping CMR shows promise in monitoring this transition.

**20 THE POLYPHENOL DELPHINIDIN INDUCES ANTIOXIDANT EFFECTS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS THROUGH ACTIVATION OF ENDOGENOUS GLUTATHIONE: IMPORTANCE OF USING RELEVANT CONCENTRATION IN IN VITRO SYSTEMS**

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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  $\geq 10$  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 emphasise 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

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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 µg) 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 5 $\alpha$ -TETRAHYDROCORTISONE EXHIBITS TOPICAL ANTI-INFLAMMATORY ACTION WITH LIMITED ADVERSE EFFECTS ON ANGIOGENESIS

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Glucocorticoids (GC) are potent anti-inflammatory drugs but have debilitating side effects. A safer alternative is required and 5 $\alpha$ -tetrahydrocorticosterone (5 $\alpha$ THB, a metabolite of the natural rodent glucocorticoid corticosterone) may provide a solution. 5 $\alpha$ THB 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 5 $\alpha$ THB on processes involved with wound repair were investigated.

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