

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