161 NORADRENERGIC RECEPTOR FUNCTION IN HEALTHY AND OBESE PERIVASCULAR ADIPOSE TISSUE

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Perivascular adipose tissue (PVAT) exerts an anti-contractile effect which is vital in regulating blood pressure. Evidence suggests that the sympathetic nervous stimulation of PVAT triggers the release of anti-contractile factors via activation of $beta_3$-adrenoceptors. There is considerable evidence of sympathetic over-activity in obesity, which could result in the loss of PVAT function, and subsequent hypertension. Therefore it was decided to examine $beta_3$-adrenoceptor function in obesity.

Electrical field stimulation (EFS) profiles of healthy and obese mouse mesenteric arteries (<200 um, +/-PVAT) were characterised using wire myography (0.1–30 Hz, 20V, 0.2 ms pulse duration, 4s train duration). To demonstrate the release of an anti-contractile factor in health, the solution surrounding stimulated exogenous PVAT was transferred to a PVAT denuded vessel. $Beta_3$-adrenoceptor function was investigated using the agonist CL-316,243 (10uM) and antagonist SR59203A (100nM). The role of the vasodilator nitric oxide (NO) was studied using nitric oxide synthase (NOS) inhibitor L-NMMA (100uM), and NOS activator histamine (100uM).

During EFS healthy PVAT elicits an anti-contractile effect ($n = 8$, $P < 0.001$); however the anti-contractile function of obese PVAT is lost ($n = 8$, $P = 0.35$). Inhibition of $beta_3$-adrenoceptors in healthy PVAT using SR59230A significantly reduced the anti-contractile effect ($n = 8$, $P < 0.01$), whereas activation of $beta_3$-adrenoceptors in obese PVAT using CL-316,243 did not restore function ($n = 7$, $P = 0.77$). Solution transfer from stimulated healthy exogenous PVAT to a -PVAT vessel significantly reduced contraction ($n = 8$, $P < 0.01$), confirming that stimulated PVAT releases a transferable anti-contractile factor. The release of this factor could be inhibited using SR59230A ($n = 7$, $P = 0.47$). Solution transfer from obese PVAT had no effect on contraction ($n = 6$, $P = 0.41$), and again could not be restored using CL-316,243 ($n = 6$, $P = 0.14$). In healthy PVAT, inhibition of NOS using L-NMMA abolished the anti-contractile effect ($n = 8$, $P < 0.01$). In obese PVAT, activation of NOS using histamine was able to restore the anti-contractile function ($n = 4$, $P < 0.05$).

These results demonstrate that in health PVAT releases an anti-contractile factor via activation of $beta_3$-adrenoreceptors, which downstream trigger the release of NO. In obesity, the anti-contractile effect is lost and cannot be restored by $beta_3$-adrenoceptor activation, but is restored by activation of NOS. This suggests that in obesity $beta_3$-adrenoreceptors must be downregulated or desensitised, leading to a loss of anti-contractile function, which may contribute to the development of hypertension.

162 POLYMERSOMES FUNCTIONALIZED WITH HSP70 –– NOVEL, SYNTHETIC CARDIOPROTECTIVE NANOVESICLES

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Background Exosomes –– nano-sized, lipidvesicles released by cells into the blood – can protect the myocardium against ischaemia/reperfusion (IR) injury.[1] This cardioprotection is mediated by heat shock protein 70 (HSP70) on the exosome surface interacting with Toll-like receptor 4 (TLR-4) on cardiomyocytes and activating intracellular protective signalling kinases.[1] Polymersomes are synthetic nanovesicles with a structural similarity to exosomes, and the capacity to function as drug delivery vehicles.

Aim The aim of this project was to develop polymersomes functionalized with HSP70 peptides as "synthetic exosomes" with a potential therapeutic application against IR injury.

Methods POEGMA-PDPA polymersomes were synthesised from hydrophilic poly[oligo (ethylene glycol) methacrylate] and poly[2-(diisopropylamino)ethyl methacrylate] blocks, and covalently functionalized with either KSTGKANKI-TITNDKGRLSK ("KST") or TKDNNLLGRFELSG ("TKD") peptides from HSP70. They were analysed using Nanosight LM10-HS nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS). Adult rat ventricular cardiomyocytes were pre-treated with polymersomes, then subjected to simulated IR. Percentage cell death was assessed using a vital dye and fluorescent microscopy.

Results In line with previously published data, pre-incubation with recombinant HSP70 protected cardiomyocytes from simulated IR injury, significantly reducing cell death from 74+-4% to 44+-1% ($P < 0.001$) with maximal protection observed at 1 ng/ml HSP70 equivalent to molar concentration of 14.3 pM. Cytoprotection was blocked in the presence of TAK-242, an inhibitor of TLR4 (83+-3%). The average size of polymersomes was ~70 nm (DLS) or 80–90 nm (NTA), and they expressed ~145 peptides per polymersome. Pre-incubation with KST- or TKD- functionalized polymersomes reduced death of cardiomyocytes exposed to simulated IR from 62+-3% to 38+-4% or 42+-4% respectively ($P < 0.001$). Significant protection was observed even at 10^8 particles /ml, representing a concentration of 0.17 pM particles, or 0.025 pM of HSP70 peptide. No protection was recorded with non-functionalized polymersomes.

Conclusion Polymersomes with HSP70-derived peptide sequences are non-toxic to cardiomyocytes and powerfully cardioprotective in a cell model of acute IR injury. Future *ex vivo* and *in vivo* experiments are required for pre-clinical assessment of these novel nanoparticles, before potential translational application.

REFERENCE

1 Vicencio *et al*. Plasma exosomes protect the myocardium from ischemia-reperfusion injury, *J Am Coll Cardiol*. 2015;**65**(2015):1525–36

163 ENDOTHELIAL CELL DERIVED EXTRACELLULAR VESICLES ENRICHED WITH VCAM-1 IN INFLAMMTION STIMULATE SPLENIC MONOCYTE MIGRATION

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Background In acute myocardial infarction (MI), monocytes are rapidly mobilised from the spleen to peripheral blood, from where they infiltrate injured tissue, with potential to contribute to both injury and repair. The mechanism by which

the injured myocardium signals splenic-monocyte mobilisation remains poorly understood. Recent work shows extracellular vesicles (EV, which carry proteins, microRNA/mRNA) are a means of rapid cell-to-cell communication, which, combined with knowledge of their composition and propensity to be taken up by other cells, suggests a possible role in signalling. Here we show that acute MI results in a net increase in circulating endothelial cell (EC)-EV that induce splenic monocyte motility *in vivo*.

Methods MI was induced in mice. EV were isolated by ultracentrifugation and analysed for size/number by Nanoparticle Tracking Analysis, western blot (EV-markers: Alix, TSG101, CD69, CD9 and Hsp70) and ELISA for EC markers (CD31, ICAM-1, P-selectin, E-selectin and VCAM-1). Primary cardiac mouse-EC (CM-EC) were used to produce EV in-vitro and were labelled fluorescently (PKH67) and transfected with cel-miR39-3p (*C.elegans*). EV were either tail vein injected into wild-type or $CD68^{GFP+}$ naïve mice and/or exposed to macrophages (RAW 264.7) *in-vitro*.

Results Acutely (24 hours) after MI there was a significant increase in circulating EV in mice ($7.6 \pm 1.7x10^8$/ml vs $3.6 \pm 1.5 \times 10^8$/ml, control,$P < 0.01$) that later subsided ($4.6 \pm 1.8 \times 10^8$/ml 4th day post-MI). Plasma EV displayed EC-surface markers, suggesting EC origin. Pro-inflammatory TNF-α ($3.0 \pm 0.1 \times 109$/ml vs $1.5 \pm 0.1x10^8$/ml control, $P < 0.01$) significantly increased EV production in CM-ECs in-vitro, whereas 'anti-inflammatory' IL-4 ($1.3 \pm 0.01 \times 10^9$/ml) and IL-6 ($1.5 \pm 0.1 \times 10^9$/ml) had no effect. Inflammatory-EC-EV displayed significant enrichment of VCAM-1 ($P < 0.05$). *In-vitro* there is a time-dependent accumulation of labelled EV in macrophages. Inflammatory-EC-EV significantly enhanced macrophage chemotaxis to MCP-1 *in-vitro* (2.2 ± 0.2 AU EV treated vs 1.7 ± 0.2AU untreated, $P < 0.01$), a response that was abolished by pre-incubating EC-EV with an anti-VCAM-1 antibody (1.8 ± 0.2AU, $P < 0.05$). Inflammatory EV significantly induced alterations in mRNA of motility genes by reducing PLEXIN-B2 ($P < 0.001$) and increasing ITGB2 ($P < 0.001$) expression in macrophages. Injected labelled EC-EV accumulate in the splenic red-pulp and injected inflammatory-EC-EV induced significant splenic monocyte mobilisation and peripheral monocytosis *in-vivo in* $CD68^{GFP+}$ naïve mice ($P < 0.05$).

In conclusion (1) *In vivo* EV are released acutely after MI; (2) Plasma-EV protein composition is consistent with endothelial cell origin. (3) Injected EV localise to the spleen and (4) mobilise splenic monocytes. (5) In culture, EV increase cell motility and (6) regulate genes that are important in motility. These findings are all suggestive of a role for EC-derived EV in monocyte activation after acute MI.

164 CARDIOSPHERE-DERIVED CELL-SEEDED POROUS COLLAGEN SCAFFOLDS FOR CARDIAC REPAIR

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Irrespective of cell type, stem cell therapy to prevent heart failure following myocardial infarction is beset by low donor cell retention. Cell loss may be prevented by immobilisation within a supporting scaffold for application across the infarct, but cells must remain viable while vasculature develops into the scaffold. Previously we have shown that cardiosphere derived cells (CDCs) can be cultured on porous collagen scaffolds (Chen *et al*, J. Tissue. Sci. Eng. 2012). Here we investigate whether CDC-seeded collagen scaffolds, cultured under hypoxia to induce release of angiogenic growth factors, can improve cardiac function when applied to the infarcted rat heart.

Methods Porous collagen scaffolds were prepared by freeze-drying a suspension of type 1 collagen (1% wt/v.in 0.05M acetic acid (pH 3.2)) on a smooth film prepared by air-drying the collagen suspension. CDCs were isolated and expanded from male Sprague Dawley rats according to published protocols (Carr *et al*, PLoS One 2011). 10^5 CDCs were seeded onto the porous collagen scaffolds and cultured in hypoxia (5% oxygen) for 7 days. Myocardial infarction was induced in female Sprague Dawley rats with reperfusion after 40 minutes, at which point the scaffold was sutured to the infarct region of the heart. Hearts (4 groups: sham, infarct, infarct+scaffold, infarct+scaffold+cells, n = 5–6 per group) were scanned in vivo using MRI at 1 week, 6 weeks and 12 weeks post MI.

Results CDC-loaded scaffolds cultured under hypoxia released significantly more VEGF than those cultured under normoxia. Bi-layer scaffolds could be sutured to the myocardium with the collagen film on the out-facing surface to reduce adhesion to the chest wall. Scaffolds without cells were attached to the infarcted heart for 2 or 6 weeks. At 2 weeks the scaffold had not fully integrated with the myocardium but by 6 weeks scaffolds were integrated and had begun to be degraded. Histological analysis showed substantial macrophage infiltration at 2 weeks and formation of blood vessels within the scaffold after 2 and 6 weeks. MRI of all four treatment groups showed that ejection fraction was significantly lower in all infarcted hearts at all time points but there was no significant difference in cardiac function between untreated infarcted hearts and those treated with scaffolds, with or without cells. By 12 weeks, in the majority of cases, no remnants of the scaffold could be detected by visual inspection.

Conclusion CDC-loaded collagen scaffolds could be attached to the infarcted heart but did not improve cardiac function. Scaffolds took time to merge with the myocardium although infiltration of macrophages and blood vessels occurred before scaffolds were fully integrated.

165 *IN SITU* EXAMINATION OF PLAQUE MACROPHAGE POPULATIONS USING MULTICOLOUR FLORESCENCE MICROSCOPY REVEALS CRITICAL DIFFERENCES BETWEEN MURINE MODELS OF EXPERIMENTAL ATHEROSCLEROSIS

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Macrophages play a central role in the development of atherosclerosis. They are highly plastic and heterogeneous cells that exhibit a spectrum of phenotypes, from pro-inflammatory or 'classically activated' to anti-inflammatory or 'alternatively activated'. Plaque macrophages in particular have roles in the pathogenesis of lesion stability and rupture, the most common cause of cardiac associated mortality. Despite considerable work aiming to characterise these phagocytic cells in disease,