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