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INHIBITION OF TUMOUR NECROSIS FACTOR ALPHA SIGNALLING IMPROVES VASCULAR REMODELLING AND DECREASES THE PRO-INFLAMMATORY AND CYTOTOXIC PHENOTYPE OF PERIPHERAL NATURAL KILLER CELLS IN A MODEL OF CHRONIC HYPERTENSION IN PREGNANCY

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Objective Pregnancy induces extensive yet relatively rapid remodelling of the cardiovascular (CV) system, however, little is known about this adaptation in women with pre-existing CV disease. We have previously characterised the stroke prone spontaneously hypertensive rat (SHRSP) as a model of deficient uterine artery remodelling and identified an increase in pro-inflammatory TNF α relative to the normotensive WKY strain during pregnancy.

Design and method SHRSP were treated with etanercept (0.8 mg/kg) or vehicle at gestational day (GD) 0, 6, 12 and 18. Animals were sacrificed at GD18. SHRSP, SHRSP treated with etanercept (ETN) and WKY (n = 6) were used for vascular studies. An independent set of animals (n = 6) were used for flow cytometry analysis.

Results Etanercept significantly reduced systolic blood pressure in the SHRSP after GD 12 (Î³SBP GD 10–21 SHRSP 12.0 ± 4.17 vs. ETN 25.8 ± 4.27 mmHg; p < 0.05). Analysis of GD18 uterine arteries showed that etanercept significantly reduced uterine artery contractile ability (SHRSP 57.3 ± 8.75 vs. ETN 35.2 ± 2.19 kPa; p < 0.01) and increased carbachol response (SHRSP 13.8 ± 3.8% vs. ETN 40.1 ± 3.25%; p < 0.05). Characterisation of uteroplacental blood flow using Doppler showed that etanercept significantly reduced resistance index relative to SHRSP (SHRSP 0.79 ± 0.02 vs. ETN 0.61 ± 0.02 resistance index; p < 0.01). Etanercept significantly increased litter size in the SHRSP (SHRSP 7.80 ± 0.44 vs. ETN 12.75 ± 0.94 fetuses), reduced resorption frequency (SHRSP 66.7% vs. ETN 25.0% dams with resorption) and decreased premature glycogen cell loss from the placenta. Further, we sought to identify the source of excess TNF α in the SHRSP. Inflammatory natural killer (NK) cells (CD3-CD161⁺) were significantly increased in the SHRSP relative to the WKY in the placenta (WKY 11.6 ± 2.39 vs. SHRSP 659.8 ± 201.2 cells/mg; p < 0.01). Etanercept reduced the percentage of NK cells which produced TNF α in the maternal circulation and placenta in the SHRSP. Additionally, etanercept significantly reduced the number of CD161⁺ NK cells in the placenta of the SHRSP (SHRSP 659.8 ± 201.2 vs. ETN 148.0 ± 12.62 cells/mg; p < 0.01) by inducing a phenotypic switch to a granzyme B_{low}, CD161_{low} population.

Conclusions Etanercept improves uterine artery function and pregnancy outcome in the SHRSP. We propose that this is through the limitation of both damaging TNF α release and cytotoxicity from NK cells.

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A HISTONE DEACETYLASE 7-DERIVED 7 AMINO ACID PEPTIDE ACTS AS A PHOSPHORYLATION CARRIER

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Histone deacetylase 7 (HDAC7) belongs to class II HDAC family, playing a pivotal role in the maintenance of endothelium integrity. There are 8 splicing variants in mouse HDAC7 mRNAs. Within the 5' terminal non-coding area of some variants, there exist some short open reading frames (sORFs). Whether these sORFs can be translated and their potential roles in cellular physiology remain unclear. In this study, we demonstrated that one sORF encoding a 7 amino acids (aa)-peptide could be translated in vascular progenitor cells (VPCs) in response to vascular endothelial cell growth factor (VEGF). The 7aa-peptide (7A) could be phosphorylated at serine residue via MEKK1. Importantly, the phosphorylated 7A (7Ap) could transfer the phosphorylation group to the Threonine residue of the 14–3–3 β protein in a cell free in-gel buffer system. The *in vitro* functional analyses revealed that 7A enhanced VEGF-induced VPC migration and differentiation toward endothelial cell (EC) lineage, in which MEKK1 and 14–3–3 β served as the upstream kinase and the downstream effector respectively. Knockdown of either MEKK1 or 14–3–3 β attenuated VEGF-induced VPC migration and differentiation. Exogenous 7Ap could rescue the effect of VEGF on the MEKK1 siRNA-transfected but not on the 14–3–3 β siRNA-transfected VPCs. The *in vivo* studies showed that 7A especially 7Ap induced capillary vessel formation within Matrigel plug assays, increased re-endothelialization and suppressed neointima formation in the femoral artery injury model, and promoted the foot blood perfusion recovery in the hindlimb ischemia model via increasing Sca1⁺ cell niche formation. These results indicate that the sORFs within the non-coding area can be translated under some circumstances and that the 7aa-peptide may play an important role in cellular processes like migration and differentiation via acting as a phosphorylation carrier.

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ENDOTHELIAL MICROPARTICLES: NOVEL REGULATORS OF VASCULAR CALCIFICATION *IN VITRO*

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Endothelial microparticles (EMPs) are complex structures with pleiotropic properties and are emerging as an index of endothelial damage; however, further work to determine the effect of EMPs on vascular smooth muscle cells (VSMC) is needed. We have shown that elevated EMPs are detected in Systemic Lupus Erythematosus (SLE) and carotid artery disease patients, who present accelerated vascular ageing and calcification. This study aims to investigate the molecular components of EMPs and whether they modulate vascular calcification and osteogenic differentiation of VSMCs *in vitro*.

EMPs were generated *in vitro* using human aortic endothelial cells (AoEMPs) by serum starvation (24 h) followed by TNF-alpha stimulation (10 ng/ml; 24 h), isolated by ultracentrifugation and quantified using flow cytometry. Human coronary artery smooth muscle cells (HCASMCs) were incubated with 10⁶ AoEMPs/ml in osteoinductive media (5 mM BGP and 2.6 mM CaCl₂) for 21 days. Calcification was assessed by alizarin red staining and calcium deposition assays. Conditioned media was collected at 7, 14 and 21 days to identify markers of bone metabolism using Bioplex array