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C3i: Poster Session - Research (1)

PS194.

Role of Erythropoietin Receptors and Ligands in Attenuating Inflammation and Apoptosis in Critical Limb Ischemia

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Objectives: Erythropoietin (EPO) exerts tissue-protective effect through a hetero-receptor complex (EPOR-CD131). The aims of this study were to: 1. Examine the expression of EPOR-CD131 in skeletal muscle obtained from human critical limb ischaemia (CLI) compared with control muscle; and 2. Investigate the tissue protective effects of EPO and a non-haematopoietic helix-B peptide of EPO (ARA 290) in an in vitro model of skeletal myotube ischemia.

Methods: Tissue samples were obtained from the gastrocnemius muscle of patients undergoing perigenicular lower extremity amputation for CLI (n=12). Control samples were obtained from patients undergoing cardiac surgery (n=12). Immunohistochemistry and western blot were used to demonstrate the expression of EPOR-CD131.

An in vitro model system of skeletal myotube ischemia was created using C2C12 myotubes in hypoxic chambers. Myotubes were pre-treated with EPO or ARA 290 before exposure to simulated ischemia. Inflammatory cytokines were measured using ELISA. Apoptosis and cell death were determined by cleaved caspase-3 assay and lactate dehydrogenase (LDH) release respectively.

Results: There was an expression of EPOR and CD131 in both ischemic and normal skeletal muscle cells with co-localization of EPOR-CD131. The expression of receptors was upregulated in CLI (p<0.01). EPO and ARA 290 attenuated the release of Interleukin-6, and reduced the cleaved caspase-3 and the release of LDH from the myotubes exposed to simulated ischemia (p<0.01).

Conclusions: Here we report the first study demonstrating expression of EPO receptors within skeletal muscle cells and elevated expression in CLI. We also show that EPO, and the non-haematopoietic helix-B peptide of EPO (ARA 290), decreases inflammation and apoptosis of ischemic myotubes in vitro. Our data suggests that the use of EPO derivatives (ARA 290) to selectively enhance the tissue protective activity of EPO may provide a novel therapeutic avenue for CLI.

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PS196.

Mitochondrial Heat Shock Proteins Regulate Vascular Smooth Muscle Cell Responses to Oxidant Stress and Determine Apoptotic Threshold

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Objectives: Resistance to apoptosis is a salient feature of neoplasia and neointimal hyperplasia. In cancer cells, a mitochondrial chaperone network consisting of Hsp-90 and its congener Hsp-75 maintains mitochondrial membrane integrity and mediates apoptosis-resistance. We hypothesize that an analogous network regulates survival in VSMC.

Methods: VSMC from human saphenous vein were treated with: growth factor (PDGF-BB), cytokine (TNF α), statins, siRNA (Hsp-75 vs non-targeting), or recombinant adenovirus (Ad-GFP control vs Ad-Hsp75). Real-time PCR and western blotting (total cell lysates and mitochondrial sub-fractions) were performed. Apoptosis was quantified by DNA content cytometry and Annexin V. Mitochondrial membrane potential (MMP) was measured after an oxidative challenge with peroxynitrite (0-1000 uM).

Results: PDGF-BB and TNF α induced upregulation of Hsp-90, Hsp-75, and their anti-apoptotic client protein survivin (SVV) within mitochondria. Pretreatment with these agonists resulted in MMP hyperpolarization and pro-

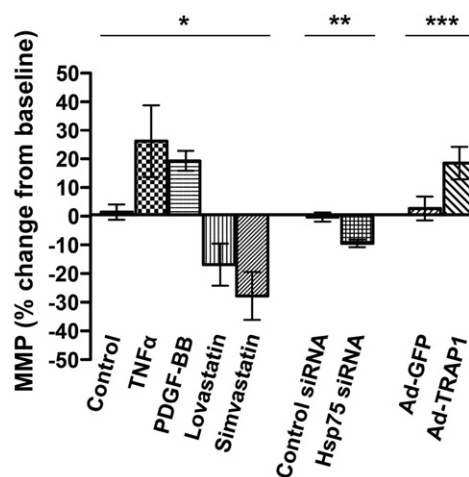


Figure. Mitochondrial Membrane Potential (MMP) in response to an oxidant stimulus with peroxynitrite (250 uM) was measured after cultured VSMC were pre-treated with cytokine (TNF α 100 ng/ml), growth factor (PDGF 100ng/ml), statin drugs (Lovastatin 100uM or Simvastatin 10uM), siRNA (control nontargeting siRNA vs Hsp75 siRNA; 200 nM), and recombinant adenoviruses (Ad-GFP vs Ad-TRAP1; MOI 500). (ANOVA *p<0.0001, student t-test **p<0.0002, student t-test ***p=0.02)

ected cells from peroxynitrite injury. In contrast, statins, at doses that caused apoptosis ($5.8 \pm 0.9\%$ versus control $1.1 \pm 0.6\%$, $p=0.003$), reduced expression of Hsp75 and SVV, and induced depolarization. Knockdown of Hsp-75 decreased MMP and sensitized VSMC to peroxynitrite, whereas overexpression of Hsp-75 increased MMP and protected cells from mitochondrial depolarization.

Conclusions: These data suggest that an organelle-specific Hsp chaperone complex regulates VSMC survival by control of mitochondrial membrane integrity. This cytoprotective mitochondrial network may be a relevant molecular target for modulation of the vascular injury response.

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PS198.

Vitamin K2 Reduces Neointimal Hyperplasia and Calcification in a Uraemia Arteriovenous Fistula Rat Model

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Objectives: Chronic kidney disease (CKD) triggers the development of neointimal hyperplasia (NIH) and calcification in arteriovenous fistulas (AVF), thus contributing to AVF failure. There is strong evidence that vitamin K-dependent proteins are involved in these pathophysiological events and it has been shown, that CKD patients suffer from vitamin-K2-deficiency. The aim of this study was to assess the impact of a perioperative vitamin K2 administration on AVF remodelling and maturation in a validated uraemia AVF rat model.

Methods: CKD was induced with an adenine rich diet. AVFs were microsurgically created in the femoral vessels. Adenine fed animals were either fed with vitamin K2 supplemented food pre- and postoperatively (preventive Group 1) or immediately after the operation (therapeutic Group 2). A third CKD group (Group 3) was postoperatively fed with normal diet. Group 4 was fed preoperatively with normal diet and postoperatively with diet supplemented with vitamin K2. Animals were sacrificed on days 21, 42 and 63 for histological and immunohistochemical analyses of the AVFs and the contralateral femoral vessels.

Results: Groups 1 and 2 presented a significant reduction ($p<0.0002$) up to 40% of the NIH formation in the fistula vein, compared to Group 3. Significant has been the

reduction of the calcification of Groups 1 and 2 ($p<0.0001$) after administration of vitamin K2, compared to Groups 3 and 4. The shrinking of the media of Groups 1 and 2 because of myofibroblast migration to the intima has been likewise significantly reduced ($p<0.0001$) compared to Group 3.

Conclusions: The results of the current study clearly demonstrate that a preventive or therapeutic vitamin K2 administration, effectively prevents both calcification and neointimal hyperplasia in fistula veins of rats with CKD.

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PS200.

Notch Activation Induces Endothelial Cell Senescence and Pro-Inflammatory Response: Implication of Notch Signaling in Atherosclerosis

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Objectives: Since Notch is essential to vascular development, we sought to determine the biologic effect of Notch signaling on mature human endothelial cells (EC) and to investigate a potential link between Notch signaling and Atherosclerosis.

Methods: Given that EC senescence and vascular inflammation are features of Atherosclerosis, we utilized in vitro loss-and-gain of function approaches to evaluate the role of Notch signaling in inducing secretion of pro-inflammatory cytokines and EC senescence. Human and murine arterial samples were evaluated for Notch expression. Notch gene profile was studied in 1179 human blood samples (from patients concurrently phenotyped for CAD by cardiac catheterization). Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0.

Results: The Notch pathway was significantly activated in aged but not young human EC. Enforced Notch signaling activation resulted in EC senescence and induced significantly higher expression of several molecules implicated in the inflammatory response (IL-6, IL-8, IL-1 α , RANTES, ICAM-1). Upregulated cytokines were specifically responsible for mediating leukocytes transendothelial migration. Several Notch pathway components were upregulated in EC at atherosclerotic lesions from human and mouse arteries. Genetic association analysis detected significant interactions between Single Nucleotide Polymorphisms (SNPs) in Notch and Notch-target genes [Notch3 x