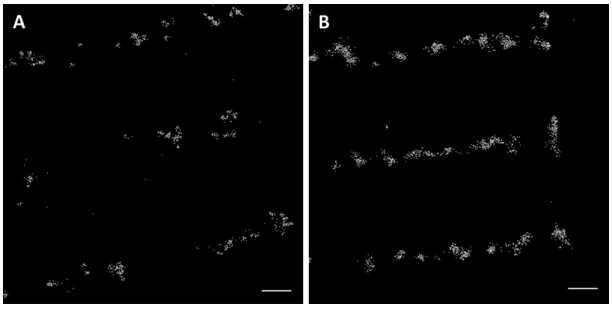
Isolated sheep ventricular and atrial myocytes were labelled with a monoclonal mouse antibody against RyR. A secondary antibody of Alexa 647 allowed image acquisition using the Nikon super resolution N-STORM 4.0 microscope. We used photo-switchable fluorescent labels and acquired blinks over 10 000 frames in order to resolve RyR clusters. In both the atria and ventricle, the RyRs were predominantly arranged along the z-lines with few longitudinal projections. However, we found distinct differences in the size and distance between these clusters. The RyRs in the ventricle are arranged into distinct clusters. In the atria, on the other hand, the RyR are more continuous along the z-line with less obvious cluster formation.

The use of super resolution allowed in-depth examination of the RyR in both the atria and ventricle. Our data suggests that there are chamber differences with respect to RyR distribution. This may account for the differences in calcium release mechanisms.



Abstract 231 Figure 1

Typical images of ryanodine receptors in sheep ventricle (A) and atria (B) obtained via super resolution STORM (stoichiometric optical reconstruction microscopy) imaging. Scale bar: 0.5 μm.

232 ENDOTHELIAL SHIP2 KNOCKOUT CAUSES NOX2 NADPH OXIDASE-DEPENDENT OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION

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Introduction Insulin-resistant type 2 diabetes mellitus (DM) leads to premature death and disability, primarily as a consequence of accelerated vascular disease. Shc homology 2-containing inositol 5 phosphatase-2 (SHIP2) is a lipid phosphatase that suppresses insulin signalling downstream of phosphoinositide-3-kinase (PI3K). Inhibition of SHIP2 has been proposed as a therapy for type II DM, but the potential impact on vascular function is currently undefined.

Methods Mice with endothelium-specific deletion of the SHIP2 catalytic domain (ECSHIP2/+) were generated by crossing mice with LoxP sites flanking exons 18–19 of the Inppl gene with Tie2-Cre mice. Pulmonary endothelial cells (PECs) were isolated from lungs using anti-CD146 microbeads. SHIP2 knockdown was studied in human umbilical vein endothelial cells (HUVECs) by transducing with SHIP2 (or control) shRNA. Immunoblotting was performed in basal conditions and after insulin stimulation, using appropriate primary antibodies. Superoxide generation was quantified using dihydroethidium (DHE) fluorescence, and endothelial nitric oxide synthase (eNOS) activity via a 13C-l-arginine to 13C-l-citrulline conversion assay (quantified as% of baseline). Aortic hydrogen peroxide abundance was assessed using an Amplex Red assay. Vasomotor function was studied *ex vivo* in aortic rings. Statistical analysis was performed with unpaired and paired t-tests assuming unequal variance, with significance defined by p-values<0.05.

Results PECs derived from ECSHIP2Ã□â€ □/+ mice had increased basal and insulin-stimulated activation of downstream signalling intermediates, including pPDK1, S473 pAKT and S1177 peNOS. Nox2 protein expression was increased (1.19 fold; p=0.04), in association with increased superoxide abundance (2.20 fold; p=0.004). This was normalised both with the Nox2 specific inhibitor Gp91ds-tat (22% reduction; p=0.05) and PI3K inhibitors, Wortmannin (35% reduction; p=0.03) and LY294002 (26% reduction; p=0.03). SHIP2 transduction in HUVECs achieved~70% knockdown. Findings were recapitulated, with more abundant S473 pAKT and S1177 peNOS. Moreover, there was enhanced Nox2 expression (1.39 fold; p=0.41) and superoxide generation (1.42 fold; p=0.02), which was suppressed in the context of Nox2 (34% reduction; p=0.03) and PI3K inhibition (39% reduction; p=0.01 [Wortmannin], 13% reduction; p=0.05 [LY294002]). eNOS activity was reduced in ECSHIP2/+ PECs treated with insulin (114% [±6] vs 136% [±3]; p=0.01). Aortic rings from ECSHIP2/+ mice had blunted insulin-mediated vasodilation, increased hydrogen peroxide abundance, and impaired vasoconstriction in response to the non-selective NOS inhibitor L-NMMA (indicating reduced NO bioavailability).

Conclusions Endothelial-specific SHIP2 inactivation causes PI3K- and Nox2-dependent oxidative stress and endothelial dysfunction. These data suggest that SHIP2 may not be an ideal therapeutic target for diabetes-associated vascular disease.

233 ANTI-ARRHYTHMIC ROLE OF SPHINGOSINE 1-PHOSPAHTE IN POST-OPERATIVE ATRIAL FIBRILLATION BY PAK1 ACTIVATION

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Background Atrial Fibrillation (AF) is the most common arrhythmia occurring post cardiac surgery. The incidence varies and depends on the type of surgery. Postoperative AF may cause hemodynamically unstable, that increase the risk of stroke and increase mortality. Current management for prophylaxis of postoperative AF is not satisfactory.

Objectives The purpose of this study is to investigate the anti-arrhythmic role of sphingosine 1-phosphate to prevent post-cardiac surgery AF.

Methods Sprague-Dawley Rats (300-350grams) Obtained from Harlan Laboratories (Udine, Italy). They were fed standard rat chow, which they had access to ad libitum. Randomly categorised into two groups n=10 each group. One group was treated with sphingosine 1-phospahte receptor agonist fingolimod (1 mg/kg, i.v) and the control group was treated with saline. Following 15 min of treatment, cardioplegic arrest with the support of cardiopulmonary bypass. ECG electrodes were

attached to limbs and using power lab charts ECG monitored and analysed. Following monitoring of rhythm for 24 hours, all animals were sacrificed and myocardial tissue was collected to analyse Pak1 protein.

Results The administration of fingolimod led to significantly better and fast recovery as compared to the control group. The episodes of AF recorded during 24 hours in the treated group was low versus the control group (p0.001). We also investigated Pak1 protein by western blot and immunohistochemistry. Expression of Pak1 observed higher in the fingolimod-treated group(p0.05).

Conclusion Sphingosine 1-phosphate receptor agonist fingolimod plays important role in prevention of AF-related to cardiac surgery by activating the Pak1 pathway.Fingolimod is FDA-approved sphingolipid that can be the potential therapeutic drug for post-operative AF prophylaxis.

Young Investigator Prize

A ENDOTHELIUM-DERIVED EXTRACELLULAR VESICLES PROMOTE SPLENIC MONOCYTE MOBILISATION IN MYOCARDIAL INFARCTION

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Background Following acute myocardial infarction (AMI), monocytes are rapidly mobilised from the spleen to peripheral blood, from where they undergo transcriptional activation and 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 AMI results in a net increase in circulating endothelial cell (EC)-EV that induce splenic monocyte motility *in vivo* and cellular transcription.

Methods Platelet-poor plasma was collected from patients with ST-segment elevation-AMI (STEMI) and mice subjected to AMI. EV were isolated by ultra-centrifugation and analysed for size/number by Nanoparticle Tracking Analysis, western blot (EV-markers: ALIX, TSG101, CD69, CD9 and Hsp70), ELISA for EC markers (CD31, ICAM-1, P-selectin, E-selectin and VCAM-1), electron microscopy and for EV-miRNAs. Human and mouse EC were used *in vitro* to evaluate EV release, injected into wild-type or $CD68^{GFP+}$ naïve mice to assess bio-distribution, splenic-monocyte mobilisation, uptake by monocytes, cellular mRNA transcription and cell motility.

Results Acutely (24 hours) after AMI there is a significant increase in circulating EV in humans ($p<0.01$) and mice ($p<0.001$) that later subsides. Plasma EV number correlates with myocardial injury in humans ($R2=0.52$, $p<0.01$). Plasma EV display EC-surface markers and show enrichment for vascular cell adhesion moleculae-1 (VCAM-1) in AMI ($p<0.05$). *In vitro* pro-inflammatory cytokines significantly increase EV production by EC, whereas 'anti-inflammatory' IL-4 and IL-6 had no effect. Inflammatory-EC-EV displayed significant enrichment of VCAM-1 ($p<0.05$). *In-vitro* labelled EC-EV accumulate in monocytes. Inflammatory-EC-EV significantly enhanced macrophage chemokineses ($p<0.05$) and chemotaxis to MCP-1 ($p<0.05$), a response that was abolished by pre-incubating EC-EV with an anti-VCAM-1 antibody ($p<0.05$). Injected labelled EC-EV accumulate in the spleen, interact with splenic monocytes and induce splenic-monocyte mobilisation and peripheral monocytosis *in-vivo* ($p<0.01$). Human plasma-EV show enrichment for 12 miRNAs in AMI, including EC-associated miR-126–3p/5p. miRNA-mRNA target gene prediction and functional enrichment analysis show roles for these miRNAs in the positive regulation of chemotaxis, cellular growth and proliferation. EC-EV significantly induced alterations in mRNA of motility genes by reducing PLEXIN-B2 ($p<0.001$), a negative regulator of motility and increasing ITGB2 ($p<0.001$) expression in monocytes.

In conclusion (1) AMI surges plasma EV; (2) Plasma-EV protein composition is consistent with EC origin. (3) Injected EV localise to the spleen and (4) mobilise splenic monocytes. (5) In culture, EC increase EV release, enhance monocyte motility and (6) regulate genes that are important in cellular movement. These demonstrate a novel role for EC-derived EV in monocyte activation after AMI.

B MITOCHONDRIAL FUNCTION REGULATES ARTERIAL AGEING IN MICE

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Background Mitochondrial DNA (mtDNA) damage is present in ageing tissues, and may promote loss of tissue function. However whether mtDNA damage or mitochondrial dysfunction is either present in the ageing vasculature or contributes to vascular ageing is unknown.

Objective To determine the time course of functional and structural changes in normal arterial ageing in mice, the effect of vessel ageing on mitochondrial function, and whether decreased or increased mtDNA damage delays or promotes vascular ageing respectively.

Methods and Results Wild-type (WT) C57Bl/6 mice were studied at 8, 22, 44 and 72 wk of age by ultrasound imaging and intra-arterial blood pressure measurements using single and dual pressure catheters to provide a range of functional parameters of arterial ageing. Vascular ageing was detected in WT mice between 22-44wk of age, with reduced carotid arterial compliance and distensibility and increased □ stiffness index (□SI), and increased aortic pulse wave velocity (PWV) (all $p<0.05$). No additional changes were noted between 44-72wk. Aortic collagen content and elastin breaks also increased by 57% and 4-fold respectively (both $p<0.05$) between 22-44wk. Similarly, mtDNA copy number assessed by quantitative PCR decreased significantly between 22-44wk