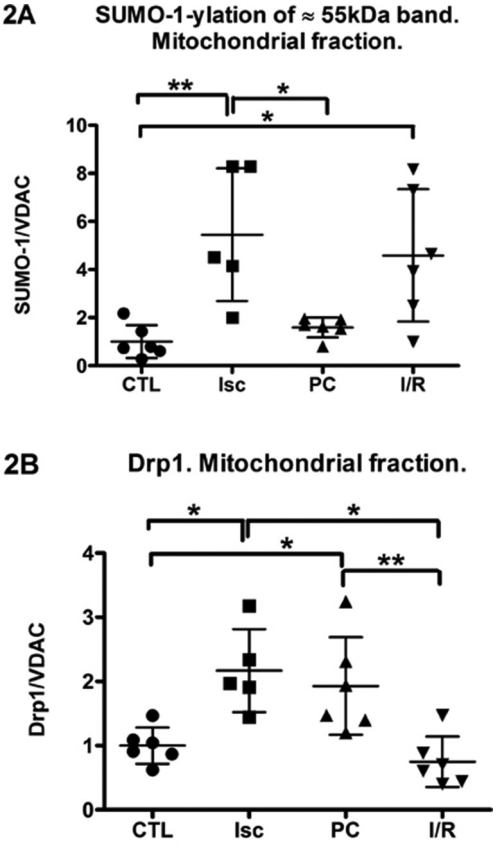
compared to both the control and preconditioning groups (Fig 2A). Intriguingly, in contrast to our finding in neurons, where mitochondrial partitioning of Drp1 decreases during ischaemia, in cardiac tissue we observed recruitment of Drp1 to mitochondria, with no change in total protein levels. Furthermore, Drp1 recruitment to mitochondria was increased by preconditioning. In the I/R group, in which cells are undergoing apoptosis, levels of Drp1 at the mitochondria are similar to controls (Fig 2B).



Abstract 229 Figure 2

Conclusion Taken together our data suggest a delicate balance between SUMOylation and deSUMOylation that regulates the recruitment of Drp1 to mitochondria. This pathway plays an important role in the vulnerability of cardiomyocytes to ischaemic damage and myocardial reperfusion injury. Interestingly, the interplay between the relevant proteins appears to differ between heart and brain cells.

230 TRACKING LATE OUTGROWTH ENDOTHELIAL CELLS IN AN ACUTE ARTERIAL INJURY MODEL

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Aim Late outgrowth endothelial cells (EOC) are strong contenders to be the true circulating endothelial progenitor cells since they are capable of clonogenic expansion, exhibit a mature endothelial phenotype, and contribute to angiogenesis *in vivo*. These cells may play a crucial role in the process of vascular repair, but whether they are able to accumulate at sites of vascular damage *in vivo* is not clear. We hypothesise that EOC, delivered locally or systemically, accumulate at, and incorporate into, a site of arterial injury.

Methods Experimental groups comprised systemic administration of the glucose analogue radiotracer ^{18}F-Flurodeoxyglucose (FDG) (Sys-Free) or FDG-labelled EOC (Sys-EOC), or local administration of free FDG (Local-Free) or FDG-labelled EOC (Local-EOC). EOC were isolated from peripheral blood from patients with coronary heart disease (n=3). Left femoral artery injury was achieved in male Sprague Dawley rats (300–350g) under general anaesthesia by inserting micro-renathane tubing via the popliteal artery. EOC were labelled with FDG (25 MBq/ml, 30 min, 37°C) and 1 million cells were administered either locally into the femoral artery or systemically via the tail vein (0.3–1.3 MBq, n=3 per group). Following injection of radiolabelled cells or free FDG, rats underwent dynamic PET scanning over 4 hours (Mediso nanoPET/CT scanner, Hungary). AuroVist and Fenestra (MediLumine Inc, Canada) were used as computed tomography vascular contrast agents. Images were analysed with PMOD software (PMOD, Switzerland) and standardised uptake values were calculated.

Results FDG radioactivity was successfully visualised by micro-PET/CT. The activity was distributed in the bladder, kidneys, heart, brain, lungs, spleen and liver (descending order). Radioactivity in the lungs was significantly higher (80 and 120 min) following systemic EOC administration compared with the other three groups (two-way ANOVA with Bonferroni post-test, $p<0.001$), and peak activity in the injured artery (55 min after administration) was significantly higher than in the non-injured right artery (two-way ANOVA with Bonferroni post-test, $p<0.001$). Following local EOC administration, radioactivity in the injured artery was significantly higher than in the non-injured right artery, or in the injured artery following local free FDG administration (two-way ANOVA with Bonferroni post-test, $p<0.001$). Radioactivity in the injured artery following local administration was considerably higher (~10 fold) than following systemic administration of either free FDG or labelled EOC.

Conclusion Preliminary analysis shows that EOC are able to target sites of vascular injury following systemic and local administration. These observations suggest that late outgrowth endothelial cells have the potential to contribute to vascular repair and regeneration.

231 SUPER RESOLUTION IMAGING UNVEILING THE DYADIC ULTRASTRUCTURE IN ATRIAL AND VENTRICULAR CARDIOMYOCYTES

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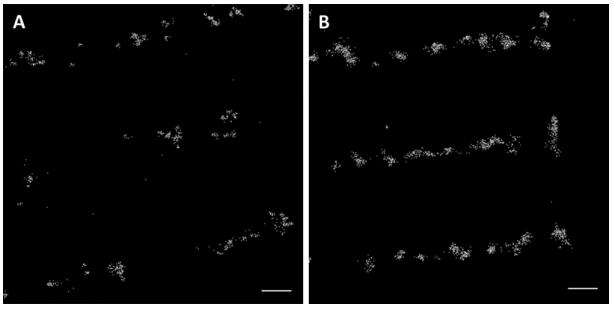
Calcium-induced calcium release drives contraction in cardiomyocytes. Located on the sarcoplasmic reticulum, ryanodine receptors (RyR) are responsible for the release of intracellular calcium stores. It has recently become apparent that the size and shape of RyR cluster may affect the functionality of that cluster, along with the relative distance to neighbouring clusters. Here, we compared the distribution of RyRs in the atria and ventricle.

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