Introduction Information on myocardial viability is important for the management of ischaemic cardiomyopathy patient. Difficulties in assessing viability arise because necrotic tissue and viable myocardium overlap at the infarct border zone. Manganese-enhanced MRI (MEMRI) is sensitive to viability and the inotropic state of the heart[1]. Mn^{2+} enters viable cardiomyocytes via Ca^{2+} channels and enhances intracellular T1 relaxation. As intracellular Ca^{2+} is a central regulator of cardiac contractility high Mn^{2+} concentration can be cardiotoxic. Here, Mn^{2+} salt has been supplemented with Ca-gluconate to provide a Calcium supplement that should overcome inotropy while still providing enhancement of viable myocardium.

Methods/Materials Two manganese-based media were used: 50 mM $MnCl_2$ in saline and 50 mM $MnCl_2$ in a 1:3 ratio with 58 mM Ca-gluconate. MEMRI experiments were performed at baseline and 7, 14, 21, 28, 35, and 42 min after intraperitoneal injection of Mn (n=4) or Mn-CaG (n=3) into adult male C57B1/6 mice using a 9.4T MRI system. Using a look-locker inversion recovery sequence[2] a series of 12 short-axis gradient echo images were acquired with the following parameters: TE=0.99 ms, TR_{ir} =~3s, TI =~100–1300 ms, FOV=25.6mm^2, DM=128^2, FA=10Ã‚Â°, slice thickness=1.5 mm. Cardiac function was assessed using cine-MRI. For T1 analysis, the average signal intensity in myocardium and blood pool regions were calculated using Segment, and were fitted to an exponential curve.

Results T1 values were evaluated at each time point and compared between two groups. Both manganese-based media rapidly led to significant shortening of T1 in the myocardium and the blood and these changes remained stable over the course of the experiment. The peak reduction of T1 in the myocardium and blood was observed slightly earlier for Mn than for Mn-CaG (figure 1a), although this difference did not reach significance in this pilot study. Image enhancement in cine-MRI was similar in the Mn-CaG group and the Mn group (figure 1b). Importantly no alterations in cardiac function or heart rate were observed and all mice recovered fully, indicating [Mn^{2+}] used was below cardiotoxic levels.

Conclusion The present study indicates that Ca^{2+} supplements to Mn^{2+} does not affect cardiac function while producing an optimum image quality. This approach has the potential of reducing the risk of toxicity of manganese-based agents and could be used to identify salvageable myocardium and monitoring new growth of heart tissue after stem cells therapy.

REFERENCES

1. Hu TCC, *et al*. (2001). *Magnetic Resonance in Medicine.*
2. Price AN, *et al*. (2011). *Journal of Cardiovascular Magnetic Resonance.*

159 TRPM2 ION CHANNEL ACTIVATION CONTRIBUTES TO REDOX-SENSITIVE VASCULAR DYSFUNCTION IN HYPERTENSION

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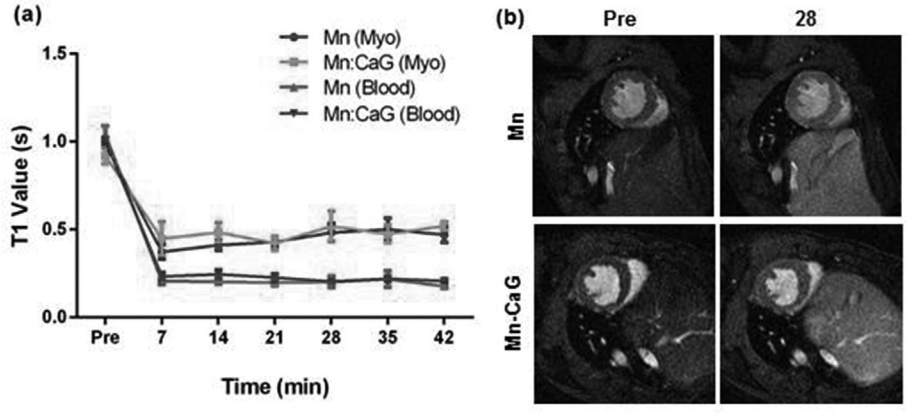
The interplay between reactive oxygen species (ROS) and Ca^{2+} plays a major role in the regulation of vascular function. However, mechanisms underlying ROS-induced Ca^{2+}-influx and signalling are not fully established. The transient receptor potential melastatin 2 cation channel (TRPM2) is a redox-sensitive cation channel that promotes influx of Ca^{2+} after activation by H_2O_2 through PARP-ADPR-dependent mechanisms in inflammatory cells. TRPM2 also regulates Na^+ influx and by increasing intracellular Na^+ content, it could interfere with the function of the Na^+-Ca^{2+} exchanger (NCX), which may confer a novel mechanism whereby ROS influences Ca^{2+} influx and signalling. Here, we postulated that redox-sensitive Ca^{2+} regulation involves TRPM2 and NCX; a process exacerbated in hypertension leading to vascular dysfunction. We also interrogated the role of Nox4 in these processes. Mesenteric arteries from wild-type (WT), LinA3 (chronic Ang II-induced mouse model of hypertension), Nox4$^{-/-}$, and LinA3/Nox4$^{-/-}$ and VSMCs cultures from human arteries were used. Vascular function, assessed by wire myography, demonstrated that mesenteric arteries from LinA3 mice present increased Phe-induced vasoconstriction (Emax – LinA3 vs WT: 9.37±0.51 *vs* 6.79±0.29); an effect ameliorated by olaparib (PARP inhibitor) and 2-APB (TRPM2 blocker). The mRNA expression of NOX4 (fold change: 3.05±0.30), TRPM2 (fold change: 1.38 ±0.24), and NCX exchanger (fold change: 1.97±0.34) were increased in LinA3 mice; an effect not observed in LinA3/Nox4$^{-/-}$ mice (a model with reduced H2O2 levels). Ang II stimulation increased Ca^{2+} influx in human VSMC from normotensive (AUC-Ex490/Em535: 15400±917.5) and hypertensive subjects (AUC-Ex490/Em535: 22460±2388). TRPM2 activation inhibitors, such as 2-APB, olaparib and 8-Br, as well as, NCX inhibitors benzamil, KB-R7943 and YM244769,



Abstract 158 Figure 1 (a) T1 value for myocardium and blood (b) End-diastolic cine-MRI images from before and 28 min after IP injection of Mn or Mn-CaG.

ameliorated Ang II-induced Ca^{2+} influx in human VSMC. In conclusion, TRPM2/NCX-induced increase in intracellular levels of calcium may be involved in hypertension-associated vascular dysfunction. Our data also suggests that oxidative stress regulates Ca^{2+} homeostasis through TRPM2-dependent mechanisms.

160 CARDIAC DYSFUNCTION IN MICE WITH REDUCED STRIATIN EXPRESSION

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Purpose Some protein kinases are regulated in STRIPAK complexes, with striatin (STRN) forming a scaffold. STRN mutations are associated with hypertension in humans and decreased expression of STRN causes arrhythmias and cardiomyopathy in dogs. Because striatin binds Ca^{2+}-calmodulin, it was thought to participate in Ca^{2+}-dependent signalling, but it is now recognised as regulatory B subunits of protein phosphatase PP2A. Thus, striatin holds the kinase in proximity to PP2A, maintaining it in an inactive state. Global striatin knockout is embryonic lethal in mice, but heterozygotes are viable and fertile. Our hypothesis is that heterozygote deletion of striatin will be detrimental to cardiac function in the context of hypertension.

Methods Cardiac function was assessed by echocardiography using a Vevo 2100 system. M-mode images of the short axis view were used for analysis of cardiac dimensions and ventricular function. Pulse-wave analysis of aortic flow was also performed. Following two baseline measurements, Strn ± mice (10–12 weeks) were infused with the pro-hypertensive hormone angiotension II (AngII; 0.8 mg/kg/day; n=5) via osmotic minipumps for 24 hour. Cardiac function and aortic flow was measured and normalised to the mean of the baseline values.

Results Compared to baseline, heart rates were elevated by 18%, whilst ejection fraction was reduced to 61% of baseline. Cardiac output was relatively preserved (87% of baseline). The internal left ventricular (LV) diameter was increased to some extent, but systolic function was severely compromised. Posterior wall thickness during systole was reduced to 79% of baseline measurements and LV internal diameter was increased by 23% giving a calculated increase in systolic volume of 67%.

Conclusions STRN plays an important role in maintaining systolic function during hypertension. Thus, the protein kinases that are regulated in striatin complexes must be significant regulators of cardiac contractility.

161 BTK INHIBITORS: FRIENDS OR FOES?

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Introduction Bruton's tyrosine kinase (BTK) plays a crucial role in the development and maturation of B-cells. A common side effect of Ibrutinib, a BTK inhibitor approved for the treatment of chronic lymphocytic leukaemia (CLL), is active bleeding in the absence of vascular injury. The mechanisms by which ibrutinib alters haemostasis however are currently unclear. The aim of this study was to investigate the effects of ibrutinib on platelet and endothelial cell function *in vitro*, to determine the mechanisms that underpin ibrutinib-induced bleeding.

Methods Platelet rich plasma (PRP) collected from healthy volunteers was treated with increasing concentrations of ibrutinib for 15 min at 37°C, prior to stimulation with collagen (2 µg/ml) or ADP (10 µM). Platelet function and activation were measured by light transmission aggregometry (LTA) and flow cytometry respectively ($CD62P^+$, $PAC1^+$) and platelet morphology analysed using scanning electron microscopy (SEM). Platelet signalling pathways were analysed by Western blotting and the generation of endothelial microvesicles (EMVs) from HUVECs enumerated by flow cytometry, following 24 hours treatment with ibrutinib (increasing concentrations from 0.1 µM to 10 µM).

Results Ibrutinib significantly reduced collagen-mediated platelet aggregation and activation in a dose-dependent manner ($p<0.05$). SEM analysis also demonstrated that collagen-mediated shape change and filopodia formation was defective following ibrutinib treatment. Consistent with these findings, signalling downstream of the collagen GPVI receptor was perturbed, with a marked reduction in PLCÎ³2 phosphorylation. Ibrutinib only exerted mild inhibition of ADP-induced platelet aggregation ($p<0.05$), which was accompanied by reduced PLA_2 activation and inhibition of VASP dephosphorylation. Additionally, our results demonstrated that at low concentrations, ibrutinib reduced the generation of pro-thrombotic EMVs, an effect that is reversed at the highest concentrations ($p<0.05$).

Conclusion Ibrutinib reduces collagen and ADP-mediated platelet aggregation, and activation by reducing phosphorylation of PLCÎ³2 and PLA_2 and inhibiting VASP dephosphorylation. In addition, ibrutinib also appears to alter endothelial cell function by reducing EMV release. Understanding the mechanisms by which ibrutinib alters haemostasis may lead to the identification of novel antithrombotic targets.

162 COMBINATORIAL ANALYSIS OF EXOME SEQUENCING DATA AND COPY NUMBER VARIANTS IN CONGENITAL HEART DISEASE PATIENTS

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Congenital heart disease (CHD) is the most common type of birth defect in humans. Most cases of CHD are sporadic with the specific interactions between genetic variants and environmental factors involved in their pathogenesis uncharacterised. Various whole exome sequencing studies have identified *de novo* mutations in different genes; however they have only explained a small percentage of CHD cases.Previous work from the group and others has identified chromosomal regions where rare copy number variants (CNVs) were significantly enriched in CHD cases compared to controls. We hypothesise that utilising available CNV data to prioritise candidate regions within which we will interrogate exome sequencing