hearts. Whether these distinct characteristics are altered differently by an ischaemic insult, thus explaining variations in vulnerability, is not presently known. This is currently under investigation.

P14 MITOCHONDRIAL REGULATION OF EXOSOMAL MICRORNA CARGO MEDIATES CELL PROLIFERATION IN SYNTHETIC VASCULAR SMOOTH MUSCLE CELLS

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In this work we aimed to study potential correlation between the synthetic hyperproliferative vascular smooth muscle (VSM) cell and exosomal-dependent signalling.

We have previously shown mitochondrial bioenergetics are upregulated in synthetic hyperproliferative VSM cells.

Total exosomal release was increased in synthetic VSM cells vs wild type (WT) cells. Total protein and RNA within exosomes in synthetic VSM was significantly greater when compared with WT VSM cells. Addition of exosomes isolated from WT or synthetic hyperproliferative cells to VSM cell cultures resulted in 4.7%±2.3% and 23.4%±4.6% respectively, $p<0.05$. qRT-PCR of exosomal contents (synthetic VSM cell vs WT VSM cells) highlighted a significant reduction in pro-apoptotic genes (Bnip3, SOD1, SOD2), a reduction in significant tumour suppressor genes (Pmaip1, p53) and significant reduction in cell cycle regulator Cdkn2a. Likewise, RNA for PI3K, 4EBP1 and mTOR were all significantly greater in exosomes isolated from synthetic VSM cell vs WT VSM cells. miRNAome sequencing highlighted significant differences in exosome contents. Notably a 10-fold increase in pro-mitogenic/synthetic miR21 and 3.5-fold loss of anti-mitogenic/synthetic miR145, synthetic VSM cell vs WT VSM cells. The use of selective anti-miRs/miR mimetics in cell proliferation assays confirmed that both miR145/miR21 are regulators of VSM cell phenotype and proliferation.

Inhibition of mitochondrial bioenergetics or mitochondrial dynamics restored the exosomal yield, exosomal contents, RNA and miRNA similar to that measured in WT cells.

Our results implicates exosomes and their miRNA contents as crucial mediators of cellular proliferation in synthetic VSM cells

P15 CYCLOSPORINE A IS PROTECTIVE AGAINST OXIDATIVE STRESS IN ADULT BUT NOT IN IMMATURE ISOLATED CARDIOMYOCYTES

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The vulnerability of intact rat hearts to ischaemia and reperfusion injury changes during post-natal development with 14 days old being most resistant. The mechanism for this is multifactorial, but one significant element is the changing characteristics of the mitochondrial permeability transition pore (MPTP). We investigated the effect of oxidative stress and Ca2+ loading on cardiomyocyte viability in cell suspension throughout development with or without MPTP inhibition.

Ventricular cardiomyocytes were isolated enzymatically from 14 (n=4), 28 day old (n=3) and adult rat heart (n=3). These cardiomyocytes were incubated with 0.5 mM H2O2 and 3 mM Ca2+ (simulated reperfusion) in the presence or absence of 2 µM Cyclosporine A (CsA). Cardiomyocytes incubated in normal buffer were used as control. Cardiomyocyte viability (assessed using trypan blue) and morphology was monitored every 30 min for 2 hours.

There was a time-dependent decrease in viability in all age groups. However, this effect was more marked in 14 day old compared to adult and 28 day old cardiomyocytes. In the adult group, CsA significantly improved cardiomyocyte viability at all time points (at 120 min, 76%±7.4 vs 53%±5.3). No protective effect was seen in 14 (22%±12.7 for CsA vs 10% ±6.6 injured control) or 28 day old cardiomyocytes (55%±7.9 for CsA vs 33±5.4 for injured control).

Unlike intact adult heart, the resistance of isolated cardiomyocytes to simulated reperfusion injury increases from 14 days of age to adulthood. The finding that CsA does not confer protection in the younger age group could be due to excessive injury or due to changes in MPTP sensitivity.

P16 HYPERPOLARIZED 13C MAGNETIC RESONANCE SPECTROSCOPY IDENTIFIES CHANGES TO MYOCARDIAL METABOLIC FLUXES IN A RAT MODEL OF DOXORUBICIN-INDUCED CARDIOTOXICITY

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Doxorubicin (DOX) is a widely used anthracycline chemotherapeutic for adult and paediatric patients and its use has greatly improved cancer survival rates. However, DOX can cause serious cardiac side effects leading to congestive heart failure. The mechanisms for this toxicity are not yet fully understood, although mitochondrial oxidative stress and altered cardiac energetics are thought to play a key role in the pathology. In this study, we measured real-time metabolic fluxes in the rat heart following DOX treatment using hyperpolarized 13C magnetic resonance spectroscopy (MRS).

Rats were treated i.v. weekly for 6 weeks with either 2 mg/kg DOX (n=12, low-dose) or saline (n=12), or for 5 weeks with 3 mg/kg DOX (n=8, high-dose) or saline (n=8). CINE MR imaging for cardiac functional analysis and hyperpolarized [1–13C]- and [2–13C]pyruvate MRS were performed at weeks 1, 3 and 6.

DOX treatment lead to a progressive and dose-dependent decrease in cardiac ejection fraction and cardiac output. Those functional changes were accompanied by reduced pyruvate dehydrogenase flux in the high-dose model and reduced 13C label incorporation into the glutamate and acetyl-carnitine pool in both models, suggesting altered citric acid cycle flux and reduced acetyl-CoA buffering capacity in the myocardium. Rats showed variability in cardiotoxic severity and the metabolic and functional changes were significantly correlated.

Hyperpolarized 13C MRS is therefore a unique non-invasive method to reveal early metabolic effects of DOX on the heart. Future research will focus on unravelling the

relationships between metabolic flux changes and the functional decline observed.

P17 CYCLOSPORIN A MEDIATED INHIBITION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE (MPTP) ATTENUATES TIOTROPIUM BROMIDE MEDIATED CARDIOTOXICITY

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Muscarinic antagonists relieve bronchoconstriction due to the progressive condition of chronic obstructive pulmonary disease (COPD). Recent meta-analyses have highlighted increased stroke and myocardial infarction with the long acting muscarinic receptor antagonist, Tiotropium bromide. Opening of the mitochondrial permeability transition pore (mPTP) triggers cardiomyocyte death, therefore modulation of the pore could promote cardiomyocyte survival.

Isolated perfused rat hearts were subjected to ischaemia/reperfusion (I/R) or normoxic protocols. Hearts were subjected to stabilisation, and perfusion ±Tiotropium (10 nM – 0.1 nM), Cyclosporin A (CsA) (200 nM) or Tiotropium (1 nM)±CsA. For I/R, regional ischaemia was induced following stabilisation. Hearts were stained using triphenyl-tetrazolium chloride (TTC) to determine infarct/risk ratios (%). Data was analysed using one-way ANOVA and LSD, presented as mean ±SEM.

All concentrations of Tiotropium significantly increased infarct/risk ratio compared with controls. CsA decreased infarct/risk with respect to controls (Normoxia: 5.1±1.0% vs 10.3±1.9%, $p<0.05$; I/R: 7.2±1.2% vs 50.9±3.9% and 10.3%±1.9%, $p<0.0001$), co-administration maintained this, with respect to Tiotropium (1 nM) in normoxia, and also with control in I/R (Normoxia: 8.4±2.1% vs 18.7±1.8%, $p<0.0001$; I/R: 16.3 ±0.8% vs 65.4±3.0% and 50.9%±3.9%, $p<0.0001$).

This is the first pre-clinical study to suggest that Tiotropium increases infarct/risk ratio in an isolated perfused heart model via mPTP opening, as CsA decreases Tiotropium- and ischaemia/reperfusion-mediated myocardial injury. These findings suggest for a role of the mitochondria in mediating the adverse cardiac side-effects seen clinically.

P18 ROLE OF cAMP in the regulation of Parkin-dependent mitophagy

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Parkinson's disease (PD) is characterised by compromised mitophagy, a highly specialised quality control process that removes dysfunctional mitochondria through a macroautophagy pathway. The proteins Parkin and PINK1 are key players in the mitophagic process. In healthy mitochondria with normal membrane potential, Parkin is located mainly in the cytosol, where its ubiquitin ligase activity is inhibited while PINK1 is imported into the mitochondria and becomes degraded by proteolysis. Following cellular stress, the depolarization of the mitochondrial membrane potential allows the stabilisation of PINK1 at the outer mitochondrial membrane (OMM), where it phosphorylates ubiquitin. This induces activation of Parkin and its translocation to damaged mitochondria, followed by mitophagy. Recent advances revealed that Parkin recruitment to depolarized mitochondria is severely inhibited by treatment with cAMP raising agents. cAMP-dependent activation of PKA has been shown to reduce PINK1 protein levels at the OMM through phosphorylation of MICOS (mitochondrial contact site and cristae organising system). Here we show that phosphodiesterase 2A2 (PDE2A2), a cAMP-degrading enzyme, interacts with components of the MICOS complex and regulates cAMP levels selectively at the mitochondria. Furthermore, our preliminary data show that in mouse embryonic fibroblasts deleted of PDE2A2 (MEF)PDE2A-/- the amount of Parkin recruited to the mitochondria is reduced compared to MEFWT under basal conditions. In agreement with these results, treatment with BAY 60–7550, a selective PDE2A inhibitor, promotes PKA-dependent phosphorylation of Mitofilin. In conclusion, we propose that PDE2A2 regulates a local cAMP pool at the mitochondria that leads to PKA-dependent phosphorylation of MICOS and Parkin recruitment to damaged mitochondria.

P19 LOW LEVELS OF THE A3243G MTDNA MUTATION IN HUMAN INDUCED PLURIPOTENT STEM CELL-CARDIOMYOCYTES DO NOT CAUSE FUNCTIONAL OR METABOLIC DISTURBANCES BUT INCREASE WITH FURTHER PASSAGING

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The heteroplasmic mtDNA mutation A3243G can cause the mitochondrial condition MELAS. Mitochondrial replacement therapy can prevent transmission of mtDNA mutations to offspring but to maintain nuclear integrity, a certain amount of cytoplasm and mutated mtDNA is carried over (<3%). It is unknown whether this will increase with age and this is particularly relevant in the heart, where mutations accumulate over time. We applied small molecule modulation of the Wnt/β-catenin signalling pathway to generate pure populations of cardiomyocytes (CMs) from human induced pluripotent stem cells (hiPSCs) from a patient with 20% heteroplasmy for the A3243G mtDNA mutation No changes in the basal beating rate or time to peak and time to 50% relaxation were found. No differences in the response to β-adrenergic stimulation by isoprenaline or muscarinic inhibition by carbachol. A3243G hiPSC-CMs showed reduced excitability (18.85±3.045 ms for control and 38.08±6.126 ms for A3243G, Mean ±SEM, $p=0.0084$) but there were no changes in other calcium handling properties. Mitochondrial DNA copy number and both mitochondrial respiration and basal glycolysis were unaffected. We have seen a gradual increase in A3243G hiPSCs and derived CMs heteroplasmy with passaging (26.4% to 38.7% over 6 passages). We conclude that A3243G heteroplasmy <40% is not sufficient to affect the generation of hiPSC-CMs and their beating, calcium handling and metabolic properties. Having observed an increase in heteroplasmy with