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.

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% heteroplasy 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 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