P017
NOVEL SYNTHETIC HSP90 INHIBITOR FORMULATION IMPROVES CARDIOPROTECTION IN RAT AND HUMAN IN VITRO ISCHEMIA/ REPERFUSION CELL MODEL

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BACKGROUND: Ischemic heart disease continues to be one of the major causes of death in the developed world despite improvements in treatment. 30% of non-lethal myocardial infarctions will develop heart failure symptoms. Improved survival of the myocardium is a need in clinical practice. Our group has previously shown that Celastrol, along with a synthetic HSP90 inhibitor analog have the potential to reduce infarct size when given as a postconditioning agent at the moment of reperfusion. The objective is to evaluate the rapid cardioprotective mechanisms of a novel formulation of the HSP90 inhibitor compound on two cell lines: rat H9c2 cardiomyoblasts and Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).

METHODS AND RESULTS: H9c2 rat cardiomyoblasts and Human iPSC-CMs were cultured. Cell signaling was evaluated by western blot to evaluate pathway activations. Both cell lines were put in ischemic conditions (no glucose, 95% N2, 5% CO2) overnight, then reperfused (normal culture) with different concentrations of HSP90i after optimizing the human iPSC-CMs' stress experiment. Cell viability and mitochondrial permeability transition pore (mPTP) opening were evaluated using assays, oxygen-free radical production by fluorescence assay and antioxidant gene messenger RNA expression via polymerase chain reaction (PCR). Results showed an increase in cytoprotective pathway activation when both cell lines were treated with 10-6M of the compound without any stress: HO-1 and HSP-70 in the first 30 minutes while AKT and ERK after 1 hour of treatment and 3 hours of recuperation. Interestingly, treatment with the compound at 10-6M at the moment of reperfusion showed decreased the viability of the cells while 10-7M improved it. Free radical production was also decreased at a concentration of 10-7M when compared to baseline, and as expected, the compound also decreased mPTP opening. These results were seen in both human and rat cell lines. Preliminary evaluation of the antioxidant gene expression in H9c2 cells only showed an increase in the expression of the cytoprotective HO-1 gene.

CONCLUSION: We have previously shown that Celastrol compounds reduce reperfusion damage in myocardial ischemia models, including myocardial infarction and donation after circulatory death. These experiments show that the effects of the novel HSP90i formulation include the expression of antioxidant genes and the launching of a series of cytoprotective pathways that stabilize the mitochondrial membrane, reduce free radical production, and improve cell survival. Further studies investigating the mechanisms further and the optimal dose are underway to fully understand the mode of action of the medication and move to animal trials.

P020
PROTOCOL DEVELOPMENT FOR SINGLE-NUCLEUS RNA SEQUENCING OF HUMAN HEART TISSUE

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BACKGROUND: Heart disease is a leading cause of global morbidity and mortality. This is partly because despite an abundance of animal and in-vitro models, it has been challenging to study human heart tissue with sufficient depth and resolution to develop disease modifying therapies for common cardiac conditions. Single-nucleus RNA sequencing (snRNAseq) has emerged as a powerful tool capable of analyzing cellular function and signalling in health and disease. Employing snRNAseq to study the human heart has the potential to unlock novel disease mechanisms and pathways. However, progress on this front has been slowed by several barriers. One key challenge is the fact that human heart tissue is very resistant to shearing and stress, making tissue dissociation and nuclear isolation difficult. Here, we describe a tissue dissociation method allowing for efficient and cost-effective isolation of high-quality nuclei from flash frozen human heart tissue collected in surgical operating rooms.

METHODS AND RESULTS: Flash frozen human cardiac tissue was obtained through an established cardiac tissue biobank. Human cardiac tissue was collected and flash frozen from operating rooms performing open heart surgery. A protocol to generate single nuclei preparations from human tissue samples was found through literature review. This protocol was trialed and adapted for flash frozen human cardiac tissue. Assessments of protocol efficacy were made via nuclear morphology on brightfield imaging, DAPI stained nuclear morphology on epi-fluorescence imaging, Tapestation analysis of RNA integrity and concentration, and snRNAseq of isolated single nuclei to assess for cell type variability. Preliminary snRNAseq results are presented. Our results show that this modified protocol can reliably dissociate human cardiac tissue and isolate a high concentration of single nuclei. The preliminary snRNAseq results of isolated single nuclei using this protocol demonstrate high cell type variability.

CONCLUSION: Our protocol addresses the challenge of nuclear isolation from frozen human hearts and allows for snRNAseq of the human heart. Preliminary snRNAseq results show that this protocol can isolate high concentrations of single nuclei and from a diverse array of cell types. This suggests that sequencing findings from nuclei isolated using this protocol may be more representative of the in-vivo cellular composition of the human heart. This paves the way for an improved understanding of the human heart in health and disease. Ultimately, this will be key to uncovering signalling pathways and networks amenable to therapeutic intervention and the development of novel biomarkers and disease-modifying therapies.