Assessing Cell-Specific Mitochondrial Response to Pediatric Cardiac Arrest

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Introduction

During cardiac arrest, the brain experiences hypoxic injury. Cardiopulmonary resuscitation (CPR) restores blood flow, but this return of circulation causes another, secondary, reperfusion injury, disrupting mitochondrial function in neurons and glial cells. However, it is not known if different cell types in the brain respond to injury differently and how these cell-specific responses vary over time after cardiac arrest. Mitochondrial health is widely accepted as an overall indicator of cellular health. This project will use a four-pronged approach to explore cell-specific responses to cardiac arrest to inform future studies targeting specific cells in post-cardiac arrest treatment.

Methods

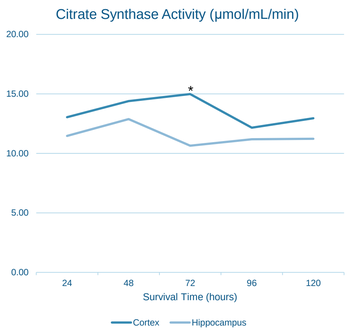
Four-week-old piglets (n = 15) underwent asphyxial cardiac arrest followed by CPR. Sham was n = 5. Following return of spontaneous circulation (ROSC), swine received protocolized post-arrest care prior to being euthanized after 24, 48, 72, 96, or 120 hours (n = 3 for each survival time). Brain was obtained for mitochondrial analysis and neuropathology. Citrate synthase activity was measured in homogenized brain tissue (cortex and hippocampus). Western blots were performed on the homogenized tissue (cortex and hippocampus) to measure relative concentrations of mitochondrial fusion- (*Opa1*) and fission-related (*Fis1*, *Drp1*) proteins. Interleukin (IL-1β, IL-6, IL-8, TNFα) concentrations in blood plasma were measured using enzyme-linked immunoassay (ELISA). Finally, cortical sections were immunolabeled with antibodies for mitochondria, nuclei, and various cell types (neurons, astrocytes, microglia, oligodendrocytes). These stained sections were then incubated in an acrylamide solution prior to undergoing gelation and expansion. Expanded tissue was visualized using confocal microscopy and mitochondria within each cell type were classified as healthy or damaged.

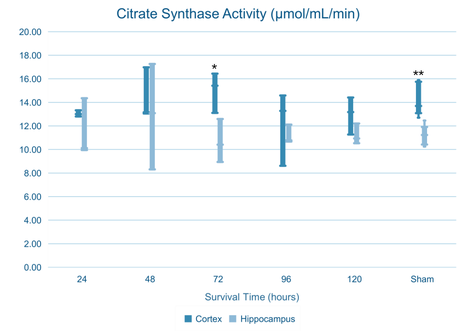
Objectives

1. Explore the molecular mechanisms of cell-specific response to cardiac arrest, enabling future research into potential targeted cell-specific treatments

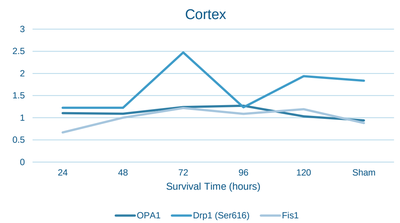
2. Establish a connection between interleukin plasma levels and mitochondrial morphology, allowing for an easy-to-use diagnostic assessment of brain mitochondrial health after cardiac arrest

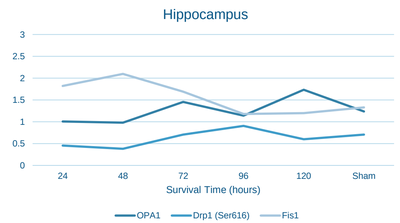
Early Findings

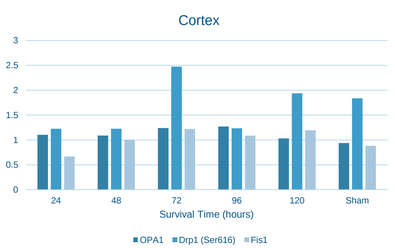


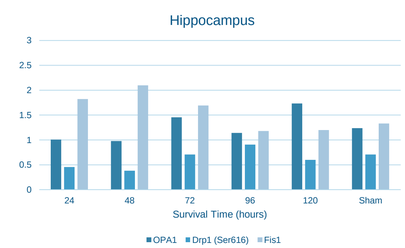


While there was a significant difference between citrate synthase activity in hippocampal and cortical tissue in the 72-hour survival group (p = 0.04), activity was not significantly different between survival times within cortex and hippocampus (p > 0.05).









At this time, not enough data has been collected to accurately classify the concentrations of *OPA1*, *Drp1* (Ser616), and *Fis1* as significantly different between survival time groups. Further data collection and statistical analysis are currently being performed.

Future Work

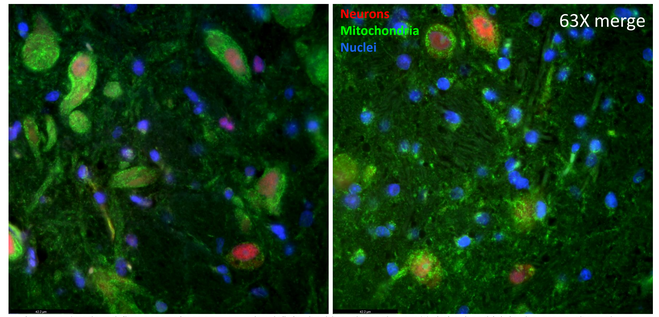


Figure 1: Overlayed fluorescent images, DAPI (nuclei) in the blue channel, Tom20 (mitochondria) in the green channel, NeuN (neurons) in the red channel. Cortical tissue from carbon monoxide-treated piglets. Images acquired at 63x magnification.

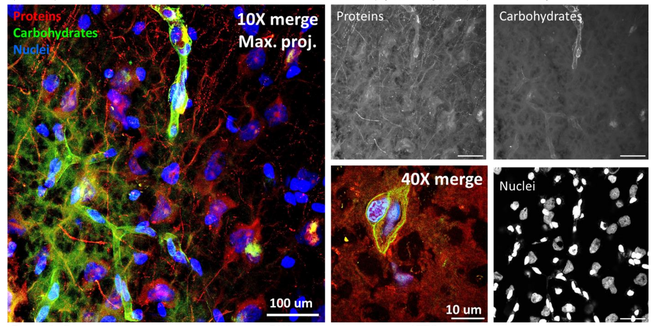


Figure 2: Overlayed fluorescent images, Hoechst (DNA) in the blue channel, ATTO hydrazide (carbohydrates) in the green channel, and ATTO NHS ester (proteins) in the red channel. ExM cortical tissue from sham piglets. Images acquired at 10x and 40x magnification.

Immunohistochemistry to classify cell-specific mitochondria as healthy or damaged was tested. However, since typical fluorescent microscopy does not provide adequate image resolution to visualize individual mitochondria (figure 1), expansion microscopy (ExM) will be employed. ExM involves physically expanding tissue in a hydrogel to achieve higher magnification. The ExM samples (figure 2) will be stained for mitochondria, specific cell types, proteins, carbohydrates, and nuclei. Individual mitochondria can be visualized at 10X magnification on a confocal microscope.

Furthermore, plasma interleukin levels will be measured to assess immune response to cardiac arrest and to develop an easy-to-use minimally invasive diagnostic assessment of brain mitochondrial health after cardiac arrest.

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