Progressive Muscle Ischemia - Could There Be A Window Of Opportunity For Using Pharmacological Agents To Limit Reperfusion Muscle Cell Death That Results Following Surgical Fasciotomy To Treat Acute Compartment Syndrome?

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INTRODUCTION: Progressive muscle ischemia results in reduced aerobic respiration and increased anaerobic respiration, as cells attempt to survive in a hypoxic environment. Acute compartment syndrome (ACS) is a progressive form of muscle ischemia that is a surgical emergency resulting in the production of Lactic acid by cells through anaerobic respiration. Our previous research has shown that it is possible to measure H+ ions concentration (pH) as a measure of progressive muscle ischemia (in vivo) (1) and hypoxia (in vitro) (2). Our aim was to correlate intramuscular pH readings and cell viability techniques with the intramuscular concentration of key metabolic biomarkers [adenosine triphosphate (ATP), Phosphocreatine (PCr), lactate and pyruvate], to assess overall cell health in a hypoxic tissue model.

METHODS: Nine euthanized Wistar rats were used in a non-circulatory model. A pH catheter was used to measure real-time pH levels from one of the exposed gluteus medius muscles, while muscle biopsies were taken from the contralateral gluteus medius at the start of the experiment and subsequently at every 0.1 of a pH unit decline. Prior to muscle biopsy, the surface of the gluteus medius was painted with a layer of 50µmol/l Brilliant blue FCF solution to facilitate biopsy orientation. A 4mm punch biopsy tool was used to take biopsies. Each muscle biopsy was placed in a base mold filled with 4% ultra-low melting point agarose. The agarose embedded tissue block was sectioned to generate 400 micron thick tissue slices with a vibratome. The tissue slices were then placed in the staining solution with Hoechst 33342, Ethidium homodimer-1 and Calcein am. The confocal microscopy images were obtained using Zeiss LSM880 confocal microscope and ZEN acquisition software (Black edition, Zeiss) in 10x magnification. The images were processed with Z-Stack and maximum intensity projection methods. The metabolic biomarkers were extracted from the snap frozen muscle biopsies and analyzed with standard fluorimetric method for ATP, PCr, lactate and pyruvate concentrations.

RESULTS SECTION: Our study shows that the direct pH electrode readings decrease with time and took an average of 69 minutes to drop to a pH of 6.0. The concentrations of ATP, PCr and pyruvate declined over time, and the concentration of lactate increased over time. At pH 6.0, both ATP and PCr concentrations had decreased by 20% and pyruvate has decreased by 50%, whereas lactate had increased 6-fold. The majority of cells were still viable at a pH of 6.0 based on viability dyes and confocal imagings (Figure 1).

DISCUSSION: Our research suggests that histologically, skeletal muscle cells are remarkably robust to hypoxic insult despite the reduction in the total adenine nucleotide pool, but this may not reflect the full extent of cell injury and quite possibly irreversible injury. The timely restoration of blood flow in theory should halt the hypoxic insult, but late reperfusion results in cellular dysfunction and cell death due to localized free radical formation. suggesting that, although this was a hypoxic model where reperfusion was not possible.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): Skeletal muscle cells are remarkably robust to hypoxic insult. Further research investigating the effects of reperfusion in vivo are warranted, as this may identify an optimal time for using pharmacological agents to limit reperfusion injury, around the time of fasciotomy to treat acute compartment syndrome.

REFERENCES:
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IMAGES AND TABLES:
Figure 1: The composite confocal image of live skeletal muscle at pH 6.0