THE EFFECT OF HYPOTHERMIA ON SKELETAL MUSCLE ISCHEMIA/REPERFUSION INJURY

+*Ward, RA; *Soudr, N; *Hunter, F; *Probe, RA; *Chaput, CD; *Chids, EW
+*Texas A&M University Health Science Center and Scott and White Memorial Hospital; Temple, Texas

rward@swmail.sw.org

ABSTRACT INTRODUCTION:
Ischemia/reperfusion injury has tremendous practical impact on clinical orthopaedic/trauma surgery in regards to limb salvage following total or subtotal limb ischemia. In clinical scenarios such as use of the surgical tourniquet, compartment syndrome, appendicular replantation, or limb ischemia associated with traumatic vascular injury, where prolonged ischemia is sometimes unavoidable, any potential protective modality that could be practically applied would be desirable. Reduction of overall tissue embarrassment caused by ischemia/reperfusion injury would be expected to lead to improved wound healing, soft-tissue flap viability, muscle contractility and fracture healing, and also, avert the development of compartment syndrome.

Much of the injury related to ischemia/reperfusion (I/R) takes place during reperfusion. Mechanisms that have been shown to play a role in I/R related tissue damage include compliment activation, neutrophil infiltration, microvascular dysfunction, mast cell degranulation, and reactive oxygen species production.1,2 Subsequent microvascular dysfunction results in tissue edema and decreased perfusion, which may lead to decreased tissue recovery.3 Research has been performed to evaluate potential modalities of attenuating the inflammatory response of this multifactorial tissue damage. Some experiments showing functional benefit involve blocking the compliment pathway.4 Inhibition of neutrophil-endothelial cell attachment by blocking intracellular adhesion molecule 1 (ICAM-1) may also have a protective effect.5,6 The effect of cooling on I/R injury has been researched and found to have a protective effect in many settings, including tissue viability and microvascular function.7,8,9,10 The use of local hypothermia dates back in medical records as far as 5000 years. It can also be applied during the ischemic phase, as opposed to pharmacologic intervention which requires reperfusion for tissue delivery.

We hypothesize that local hypothermia is protective against microvascular permeability following I/R injury in skeletal muscle. Additionally, a novel model was utilized for evaluation of microvascular permeability in fast-twitch skeletal muscle in the setting of ischemia/reperfusion (I/R) injury.

METHODS:
Surgical procedures were conducted at The Texas A&M University Health Science Center College of Medicine, Medical Research Building, after approval by the Animal Care and Use Committee. This facility is approved by the American Association for Accreditation of Laboratory Animal Care in accordance with National Institutes of Health guidelines.

Fifteen male, Sprague-Dawley rats weighing between 250 and 350 grams were randomized into three groups of five. Extensor digitorum longus (EDL) muscles were dissected for intravital visualization of 30-40nm post-capillary venules, and an intravenous bolus of 100mg/kg FITC-albumin in lactated ringsers was given.

Sham animals underwent a mock ischemic period for one hour without application of the tourniquet followed by a one-hour mock reperfusion period, with fluorescence images obtained every ten minutes. Warm and Cold I/R animals had the tourniquet applied for a one-hour period followed by a one-hour reperfusion period with the EDL maintained at 34° or 10°C, respectively. Images were analyzed for the ratio of fluorescence intensity in the interstitium to the intravascular space as a measure of vascular leak.

The EDL from the experimental extremity and from the contralateral, control extremity were then harvested, and stored at -80°C, for later wet-dry weight analysis. The ratio of wet to dry weights was calculated for each sample.

The data were analyzed to determine significance between groups by analysis of variance. When significance was found, post hoc t tests were performed to determine the site of significance within the data sets. The differences were considered significant at a value of p<0.05. All data are presented as means +/- standard error.

RESULTS:
The ratio of interstitial to intravascular fluorescence intensity of Sham animals showed very little change over the duration of the experiment (delta I max 1.12, +/- 0.03). In Warm I/R animals, the change in fluorescence intensity ratio trended upward throughout the reperfusion period (delta I max 2.71, +/- 0.12). This marked a significant increase in permeability compared to the Sham group (p-value < 0.001). In Cold I/R animals, fluorescence intensity ratios progressed in a similar pattern to Sham animals throughout the reperfusion period (delta I max 1.27, +/- 0.10). There was a significant difference in the Cold I/R group compared with the Warm I/R group (p-value < 0.001), but not when compared to the Sham group (p-value = 0.17).

On wet to dry weight analysis, a similar trend was seen, but statistical significance was not achieved with the sample size presented. The experimental to control wet-dry ratio for the Sham group was 0.71 +/-0.17. This ratio trended upward in the WarmI/R group to 1.38 +/- 0.68 (p-value 0.39). However, in the ColdI/R group, the ratio trended slightly downward to 0.51 +/-0.09 (p-value 0.36).

DISCUSSION:
The data obtained using this model supports the hypothesis that local hypothermia is a non-pharmacologic protective mechanism for fast-twitch skeletal muscle from increased microvascular permeability following I/R injury. This model could be valuable in establishing various mechanisms responsible for the protection afforded by hypothermia. Additionally, intravital microscopy may provide an earlier and more direct measure of microvascular permeability, in this setting.

REFERENCES:

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