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Survival benefits of remote ischemic conditioning in sepsis

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ABSTRACT

Background: Sepsis remains the leading cause of death in the surgical intensive care unit. Prior studies have demonstrated a survival benefit of remote ischemic conditioning (RIC) in many disease states. The aim of this study was to determine the effects of RIC on survival in sepsis in an animal model and to assess alterations in inflammatory biochemical profiles. We hypothesized that RIC alters inflammatory biochemical profiles resulting in decreased mortality in a septic mouse model.

Materials and methods: Eight to 12 week C57BL/6 mice received intra-peritoneal injection of 12.5-mg/kg lipopolysaccharide (LPS). Septic animals in the experimental group underwent RIC at 0, 2, and 6 h after LPS by surgical exploration and alternate clamping of the femoral artery. Six 4-min cycles of ischemia-reperfusion were performed. Primary outcome was survival at 5-d after LPS injection. Secondary outcome was to assess the following serum cytokine levels: interferon-γ (IFN-γ), interleukin (IL)-10, IL-1β, and tumor necrosis factor-alpha (TNFα) at the baseline before LPS injection, 0 hour after LPS injection, and at 2, 4, 24 hours after induction of sepsis (RIC was performed at 2 h after LPS injection). Kaplan–Meier survival analysis and log-rank test were used. ANOVA test was used to compare cytokine measurements.

Results: We performed experiments on 44 mice: 14 sham and 30 RIC mice (10 at each time point). Overall survival was higher in the experimental group compared to the sham group (57% *versus* 21%; $P = 0.02$), with the highest survival rate observed in the 2-hour post-RIC group (70%). On Kaplan–Meier analysis, 2-h post-RIC group had increased survival at 5 days after LPS ($P = 0.04$) with hazard ratio of 0.3 (95% confidence interval = 0.09-0.98). In the RIC group, serum concentrations of IFN-γ, IL-10, IL-1β, and TNFα peaked at 2 h after LPS and then decreased significantly over 24 hours ($P < 0.0001$) compared to the baseline.

Conclusions: RIC improves survival in sepsis and has the potential for implementation in the clinical practice. Early implementation of RIC may play an immune-modulatory role in sepsis. Further studies are necessary to refine understanding of the observed survival benefits and its implications in sepsis management.

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Introduction

Sepsis is a systemic inflammatory response to an infectious stimulus, with deleterious consequences which range from alterations in physiological parameters to multisystem failure and death.[1,2] Each year, over 750,000 surgical patients in the United States are affected by sepsis which is one of the leading causes of death in surgical intensive care units.[3] Source control with antibiotic therapy has been the primary modality of treating sepsis. However, in recent years, treatments focusing on modulating the inflammatory response have gained interest.[4-6] Although various treatment modalities (steroids, prostaglandins, beta-blockers, leukotriene inhibitors) have been identified as potential treatment options for sepsis, none of them stands out as definitive, leaving a void for further research.

Remote Ischemic Conditioning (RIC) is a novel treatment modality in which normal tissues are subjected to short cycles of ischemia followed by reperfusion resulting in reduction of an ischemic injury at a remotely injured site. RIC has been shown to improve outcomes and survival in both animal and human models after myocardial infarction, transplantation, and elective neurosurgical procedures.[7-9] It is thought to work by releasing endogenous anti-inflammatory mediators rendering global protection to the body against subsequent ischemic insults.[10] However, the therapeutic efficacy of RIC in sepsis remains unknown.

The aims of this study were to determine the survival benefit, optimum timing, and immune-modulatory role of RIC in a septic mouse model. We hypothesized that RIC alters inflammatory biochemical profiles resulting in decreased mortality in a septic mouse model.

Methods

Animals

Male C57BL/6J (8-12 weeks) mice were acquired from the Jackson Laboratory, ME and were maintained in a pathogen-free environment with access to food and water *ad libitum*. Animals were allowed to acclimatize to the environment for 5 days prior to the initiation of experimental protocols. Experimental protocols were designed to minimize discomfort to the animals and were powered to the number of animals required to assess for significant difference in our primary outcome (power = 80%, alpha = 5%). All experiments were conducted in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines at the University of Arizona.

Septic model

Sepsis was induced by intra-peritoneal injection of lipopolysaccharide (LPS) (E.coli type O111:B4; Sigma, St Louis, MO) in the animals. Animals were anaesthetized using 3% isoflurane and then 12.5-mg/kg LPS diluted in 1 mL of saline was injected intraperitoneally. Based on our previously established and published septic mouse model, this dose of LPS has a mortality of 90% at 120 h.[11]

Experimental protocol

Survival and optimal time point

After induction of sepsis, 44 animals were randomly assigned into two groups: experimental (30 mice total; 10 at each time point) or sham (14 mice; Fig. 1). In the experimental group, remote ischemic conditioning was performed at 0 h, 2 h, or 6 h after induction of sepsis.

Surgical procedure RIC

For the animals in the experimental group, the surgical site was clipped of hair and cleansed with povidone-iodine and 70% isopropyl alcohol. The right femoral artery was identified and isolated after dissection of the surrounding structures. RIC was performed by occlusion of the femoral artery using a microvascular clip. Six cycles, 4 minutes each, of femoral artery occlusion (ischemia) (clamp-on) followed by reperfusion (clamp-off) were performed at 0 h, 2 h, or 6 h after induction of sepsis.

Surgical procedure sham

A sham procedure was performed in the sham group after induction of sepsis. The animals in the sham group underwent surgical procedure identical to that of the experimental group except that the femoral artery was not occluded.

All the animals were monitored for mortality every 12 h for up to a 120-h period. Animals surviving at 120 h were euthanized. The primary outcome measure was survival at 120 h after induction of sepsis. Induction of sepsis was defined as intra-peritoneal injection of LPS.

Inflammatory profile

For analysis of inflammatory profile, a group of 15 new mice were randomized into five groups (three mice in each group). In the control group, sepsis (LPS injection) was not induced, and mice were euthanized after performance of the sham surgical procedure. In the remainder of the 12 mice, sepsis was induced by using the same methods as described above. Three mice were euthanized at 0 h after induction of the sepsis and performance of sham surgical procedure. In the remaining nine mice (experimental groups), RIC was performed at 2 h after induction of sepsis. Mice were then euthanized at 2 h, 4 h, and 24 h after LPS injection. Blood samples in all the mice were collected by direct cardiac

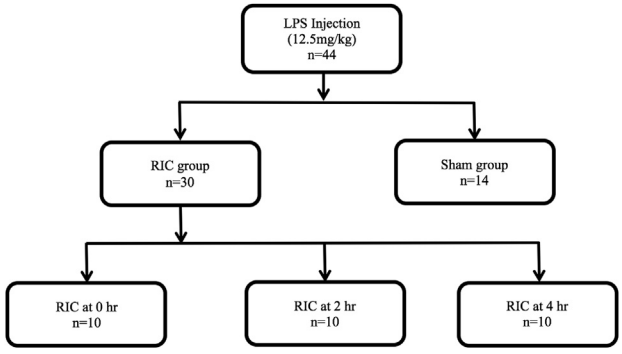

Fig. 1 – Details of the study model for survival.