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Effect of hypothermia on splenic leukocyte modulation and survival duration in severely septic rats

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ABSTRACT

Background: Therapeutic hypothermia (HT) in severe septic shock is associated with prolonged survival. We hypothesized that moderate HT would prolong survival and modulate the inflammatory response in rats with septic shock by exerting its therapeutic effect on splenic leukocytes.

Materials and methods: Severe septic shock was created in rats by cecal ligation and incision (CLI). One hour after CLI or laparotomy, rats were randomized to sham, normothermia (NT), or 4 h of HT followed by 2 h of rewarming. HT (31 ± 1°C) was induced using a cooling blanket and monitored via a rectal temperature probe.

Results: Survival duration was 2.78 ± 1.0 h in NT rats and 8.33 ± 0.32 h in HT rats ($n = 8$/group, $P < 0.0001$). In separate groups, 3 h after CLI, the spleen weight was significantly smaller in NT rats (769 ± 100 mg) than in HT rats (947 ± 157 mg, $P = 0.04$). Fluorescent immunostaining of formyl peptide receptors on leukocytes in spleen tissue showed considerably higher formyl peptide receptor expression in HT rats than in NT rats. Significantly elevated proinflammatory cytokines and myeloperoxidase enzyme in plasma were found in NT rats compared with HT rats. Anti-inflammatory cytokine, interleukin-10, was significantly higher in HT rats. Both proinflammatory cytokines and plasma myeloperoxidase were significantly reduced in splenectomized NT rats.

Conclusions: Moderate hypothermic therapy significantly prolongs the survival duration of rats with severe septic shock. HT dampens the inflammatory response during septic shock by modulating the spleen to an anti-inflammatory mode and preventing the spleen from releasing activated splenic leukocytes into the blood.

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Introduction

Sepsis has become a major public health concern, and its incidence is increasing.[1] Infection-induced sepsis is a leading cause of mortality and critical illness worldwide.[1,2] An initial sepsis-related inflammatory response may quickly develop into septic shock and multiple organ dysfunctions—a leading cause of morbidity and mortality in these critically ill patients.[3,4] The current treatment of septic patients mainly consists of source control, broad-spectrum antibiotics, and overall supportive care. This treatment protocol has been essentially unchanged over the past decade.[3] The Surviving Sepsis Campaign, published in 2012, advocates early and aggressive fluid resuscitation, empiric antibiotics, identifying the source of infection, and targeted therapy when applicable.[5]

The concept of therapeutic hypothermia (HT) during septic shock was advocated over 40 y ago when Blair *et al.*[6] first described its therapeutic use in 1961. Since then, there have been multiple studies supporting and/or condemning the use of therapeutic HT.[7-12] In experimental studies, therapeutic HT is protective against inflammatory responses and improves survival in animals with severe septic shock.[13] Recent studies have shown that splenectomized animals that undergo cecal ligation and incision (CLI) have improved survival compared with their intact counterparts.[14,15] Spleens harbor inflammatory cells such as T and B cells, monocytes, and neutrophils. When the body detects a bacterial load in the bloodstream, the spleen is stimulated and plays a key role in clearance of bacteremia.[16,17]

In the current study, we sought to identify the role of moderate HT in the treatment of severe septic shock induced by CLI in rats. The role of the spleen in mediating the inflammatory responses during severe septic shock and the effect of moderate HT on the spleen were investigated.

Materials and methods

This study conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Eighth Edition, revised 2011) and was conducted under protocols approved by the University of Virginia's Institutional Animal Care and Use Committee.

Animals

Adult male Sprague–Dawley rats (13-15 wk old, Charles River Laboratories International, Inc, Wilmington, MA) were used in the study. The rats were housed in a controlled environment with free access to food and water. They had a minimum of 7 d after arriving to become adjusted to their surroundings in accordance with protocol. All animals underwent either CLI or sham surgery (laparotomy only).

CLI procedure

We used a severe sepsis model based on a previous study designed by Rim *et al.*[13] Given previous experiences with rat survival time, we used a CLI model targeting 100% mortality within 12 h in the control group. Animals were placed in an induction chamber, and general anesthesia was initiated with 3% isoflurane. Once fully anesthetized, an intramuscular injection of ketamine-HCL (50 mg/kg) and xylazine-HCL (10 mg/kg) was administered, with a repeat dose administered 4 h later.

Under sterile conditions, a 2-cm vertical midline incision was made through the abdominal wall, and the cecum was carefully extruded. The blind-ending distal half of the cecum was ligated with 4-0 silk suture (Ethicon, Inc, Somerville, NJ), and a 0.5-cm incision was created along the antimesenteric portion of the cecum. Figure 1 illustrates the CLI procedure. The cecum was gently manipulated so stool spilled into the abdomen, and 5 mL of sterile saline was flushed into the abdominal cavity to ensure diffusion of the stool. Abdominal fascia and skin were closed in two layers with running 5-0 Polysorb sutures (Medtronic, Minneapolis, MN). We then gently massaged the abdomen to stimulate a diffuse peritonitis. The rats were then resuscitated with a dorsal subcutaneous injection of normal saline (warmed to 37°C) between the shoulders at a dose of 5 mL/100 g body weight. Additional fluid (same dose and temperature) was administered at 3 and 5 h following CLI. Additionally, the rats were administered SQ buprenorphine (0.05 mg/kg) for pain control at the conclusion of the surgical procedure.

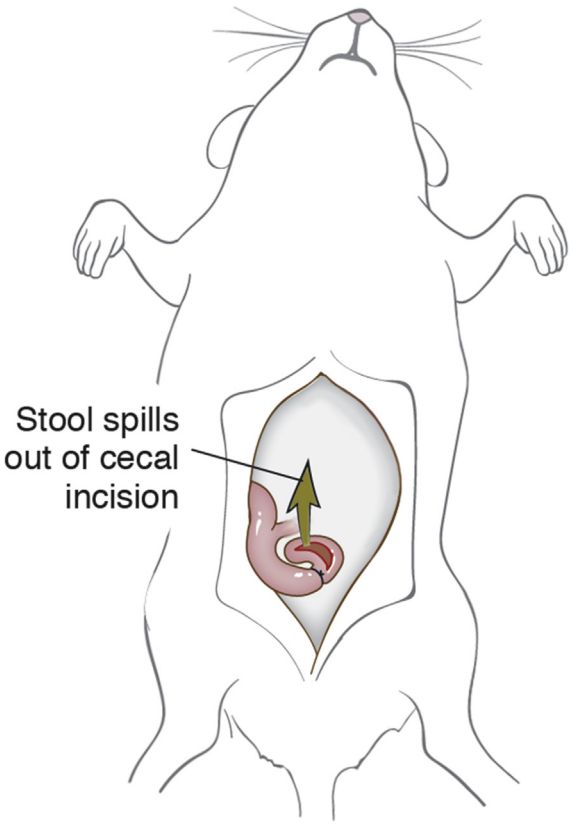

Fig. 1 – Illustration of cecal ligation and incision procedure through vertical midline laparotomy (created by Anita Impagliazzo). (Color version of figure is available online.)