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Hypothermia predicts the prognosis in colon ascendens stent peritonitis mice

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ABSTRACT

Background: The mortality rate of severe sepsis remains unacceptably high. It is difficult to make advances in the treatment of this problematic and increasingly frequent medical condition. In severe sepsis, hypothermia can be recognized as an important feature. The present study investigated the role of hypothermia in the prognosis of the colon ascendens stent peritonitis (CASP) model.

Methods: We employed the CASP model for wild-type C57BL/6 mice. We compared physiologic indices in survivor and non-survivor groups after CASP to test whether low temperature might be a helpful predictor in sepsis. To certify this hypothesis, we examined the survival rate, peritoneal leukocytes, and organ damage. We also measured the bacterial burden and inflammatory cytokine levels at different times.

Results: The temperature varied dramatically in the survivors' group compared with the non-survivors' group at 18 h. We divided the CASP models into a mild group and a severe group, based on temperatures above or below 32°C at 18 h. Mice in the severe group had a lower survival rate (0% *versus* 87.5%), more peritoneal leukocytes, more bacterial culture results, higher expressions of cytokines, and more classical features in pathology compared with the mild group.

Conclusions: Hypothermia (below 32°C at 18 h) might be a predictor of prognosis in CASP-induced sepsis.

1. Introduction

Sepsis represents a clinical syndrome resulting from the systemic inflammatory response to bacterial infection. The pathogenesis of sepsis involves a complex cellular activation process of neutrophils, monocytes, and microvascular endothelial cells, which results in the release of proinflammatory mediators, the activation of the complement and coagulation cascades, and the activation of fibrinolytic systems [1]. Widely publicized evidence-based guidelines have helped clinicians manage patients with severe sepsis. However, the mortality rate from severe sepsis remains unacceptably high, and it is difficult to make advancements in the treatment of this important and increasingly frequent medical condition [2].

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The high mortality rate of sepsis may result from the failure of the initial pathogenic clearance mechanisms or from susceptibility to a secondary infection, both of which result in tissue and organ damage, in the context of a spiraling clinical picture [3].

The colon ascendens stent peritonitis (CASP) mouse model mimics surgical patients with anastomosis leakage, by introducing a stent into the ascending colon, which allows the intestinal leakage of bacteria into the peritoneal cavity [4]. It represents a murine model for diffuse peritonitis, in which a variety of murine intestinal floras give rise to a polymicrobial infection. Bacteriemia and serum cytokine (including both proinflammatory and anti-inflammatory) levels develop rapidly in the CASP model. This rapid concomitant release of proinflammatory and anti-inflammatory mediators represents a central feature of the inflammatory response at the onset of sepsis [5,6].

Sepsis and its main complication, septic shock, induce several physiological alterations that include changes in blood pressure, body temperature, and hormone secretion. Among these, hypothermia is a well-known thermoregulatory disorder and a significant problem in the clinical management of sepsis to date [7,8]. Hypothermia can cause early increased expression of systemic interleukin (IL)-6 and IL-10, but does not induce significant changes in tumor necrosis factor-α (TNF-α) and IL-1β expressions in endotoxemic mice [9].

As previously reported, hypothermia may appear at the early stage of septic condition and my represent an indicator for a lethal outcome in other septic models [10,11]. However, it is unknown whether hypothermia might be a predictor of prognosis in the CASP model.

In the current study, we attempted to determine the role of body temperature as an indicator of the prognosis of sepsis in the CASP model. We showed that early (at 18 h) detection of hypothermia can be a prognostic of mortality, which suggests an individualized, prognostic-based approach as a potentially new strategy in the treatment of sepsis.

2. Materials and methods

2.1. Animals

We bred C57BL/6 mice at the animal facility of the Department of Experimental Medicine, Chinese Academy of Medical Sciences. We used male mice aged 8–12 wk and weighing 20–23 g throughout the experiments. They were maintained under a 12-h light/dark cycle, at 24°C ± 2°C and were fed a standard diet and double-distilled water. We performed all experimental procedures according to the regulations of the administration of affairs concerning experimental animals, set out by the China Legal System Publishing House (1988).

2.2. Surgical procedure for the CASP model

We performed the CASP animal model surgically under sterile conditions, as described in a previous report [4]. During complete anesthesia and after disinfecting the abdomen, we opened the abdominal wall through a 1-cm midline incision. After exposing the ascending colon, we introduced an 18-gauge silicon sterile tube through the intestinal wall into the lumen of the ascending colon for about 1 mm, and then fixed it with two stitches. Then, we milked stool from the cecum into the ascending colon and the stent until a small drop of stool emerged from the stent. We flushed 0.5 mL sterile saline solution into the peritoneal cavity before closure. We performed sham operations without puncturing the colonic wall. During the surgery and postoperative recovery, we placed the animals on a thermal pad at 37°C. We determined the temperature using a thermometer probe (Precision; Harvard, Holliston, MA) inserted approximately 5 cm for at least 10 s until the results became stable.

2.3. Temperature monitoring

We evaluated the effects of sepsis on the overall physical condition of CASP model by determining the temperature every 6 h, starting from induction of anesthesia before the surgical procedure to 72 h post-surgery. We measured the temperature using a thermometer probe inserted orally.

2.4. Samples

We killed animals at 6, 18, and 24 h after surgery. In each group, we examined at least four mice for each time point. We collected the blood, spleen, and peritoneal content under sterile conditions, and obtained blood samples by puncturing the retro-orbital plexus. We collected peritoneal liquid intra-abdominally. The abdomen was opened through a midline incision after thorough disinfection and without injuring the muscle under antiseptic condition. We injected 4 mL sterile saline solution into the peritoneal cavity using a sterile syringe and then aspirated it out of the peritoneal cavity twice. We harvested the spleen and dissected it in a sterile plate with phosphate-buffered saline. We collected spleen cell suspensions by mincing and pressing them gently through a stainless-steel mesh.

To investigate the local immune response in the peritoneal cavity, we counted the number of leukocytes within the peritoneal cavity and stained the peritoneal liquid with Giemsa.

We also harvested livers, lungs, and ascending colons for hematoxylin-eosin (HE) staining.

2.5. Bacterial cultures

To determine systemic and local bacterial spread in the mild and the severe groups, we grew bacterial cultures from blood, spleen, and the peritoneal cavity at 6, 18, and 24 h after surgery.

We plated aliquots of serial log dilutions (1:10, 1:100, and 1:1000) of cell-free peritoneal fluid, spleen suspensions, and blood on Columbia sheep–blood agar. We cultured plates under aerobic conditions at 37°C and counted colonies after overnight incubation. Bacterial counts are expressed as colony-forming units per organ, per milliliter of peritoneal lavage, or per milliliter of blood. For identification, we conducted bacterial counts in accordance with routine bacteriological methods.

2.6. Cytokines and chemokines

We detected several cytokines and chemokines, including TNF-α, IL-6, IL-10, and monocyte chemotactic protein-1