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Original Contribution

Accompanying mild hypothermia significantly improved the prognosis of septic mice than artificial mild hypothermia

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ABSTRACT

Background: Some patients with sepsis are found with accompanying mild hypothermia (ACMH); however, the effects of ACMH on the patients with sepsis are poorly understood.
Objective: To compare the impacts of ACMH and artificial mild hypothermia (ATMH) on mortality, systemic inflammatory reactions, and organ functions in mice with sepsis.
Methods: Septic mouse models were induced and divided into ACMH, un-hypothermia, keep normothermia, and ATMH groups, according to the anal temperature and the thermic intervention strategy. The mortality rate, serum levels of tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), and interleukin (IL)-4 and liver and renal functions of the mice in each group were recorded. Liver, lung, and renal tissues of the mice were stained and examined under optic microscope.
Results: The mortality rate in the ACMH group was the lowest among all the sepsis groups. Increased serum levels of TNF-α, IFN-γ, and IL-4 and impairments of the liver and renal functions were found in the septic mice. The serum levels of TNF-α, IFN-γ, and IL-4 were significantly lower and the liver and renal functions of ACMH group were not impaired significantly as compared with other sepsis groups. Pathological examinations of the lung, liver, and renal tissues showed that the ACMH group were with the lowest pathological score among all the sepsis groups.
Conclusion: Accompanying mild hypothermia and ATMH could both reduce mortalities in mice with sepsis, and ACMH could reduce mortality even lower, and more alleviate systemic inflammatory responses and the damages in lung, kidney, and other organs were lighter.

1. Introduction

Hypothermia, a protective treatment method for acute brain damage that has been considered as the only effective method in the 20th century, has now been popularly accepted in clinical practices [1–5]. Hypothermia therapy could reduce the bleeding and edema of blood vessels, prevent the infiltration of neutrophils, reduce the release of excitatory neurotransmitter, lower the intracellular calcium accumulation, prevent the generation of oxygen free radicals, decrease the expression of cytokines, and reduce the cellular apoptosis [6]. Studies in recent years have demonstrated that a mild hypothermia therapy could significantly reduce the mortality rate of sepsis [7–11] from different aspects including proinflammatory and anti-inflammatory factors [7], cell apoptosis [6], and functions of autonomic nerves and different organs [8–11].

Sepsis is a systemic inflammatory response syndrome caused by an infection. The pathophysiologic processes of sepsis involved multiple cytokines and inflammatory factors. Despite the improvements of novel anti-inflammatory treatments and organ function–supporting techniques, sepsis is still one of the most important causes of death for the critically ill patients [12–15]. Interestingly, we found clinically that some septic patients would also appear the status of accompanying hypothermia, among whom some might exhibit the status of accompanying mild hypothermia (ACMH), but there still existed controversies about whether keeping normothermia should be performed toward this kind of accompanying hypothermia [16,17]. Few studies have investigated if the strategy is appropriate, the changes of organ functions of the patients under normothermia or hypothermia, the effects of accompanying hypothermia on the patients with sepsis, and the differences of the efficacies as comparing with artificial mild hypothermia (ATMH). The aim of this study were examined to investigate whether the sepsis accompanied mild hypothermia could result in protective effects on the body and the possible mechanisms involved; in addition, which one between ACMH and ATMH could better improve the prognosis of the mice was also investigated.

2. Materials and methods

Endotoxin plays a very important role in the pathogenesis of sepsis caused by the gram-negative bacterial infections; in addition, lipopolysaccharide (LPS) could also induce hypothermic reactions in rodents [18,19]. Hence, in the present study, intraperitoneal injection of LPS

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was performed to induce sepsis in BALB/c mice [20–23], and then the mice were divided into different groups, namely, ACMH, unhypothermia (UH), keep normothermia (KN), and ATMH groups, according to the anal temperature and the thermic intervention strategy [8–11]. The mortality rate in each group was evaluated; the levels of tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), and interleukin (IL)-4 were measured; and the changes in the renal and liver functions, as well as the pathological changes in the liver, lung, and renal tissues, were examined.

2.1. Animals, reagents, and equipment

Specific Pathogen Free grade BALB/c mice (6-8 weeks old) were purchased from the Laboratory Animal Centre of Anhui Medical University, Anhui Province, China (scxk [Wan] 2011-002]. The present study was approved by the Ethics Committee of Anhui Medical University (No. LLSC2013028), China, and the usage of the animals and the procedures were performed in accordance with the guidelines issued by the Ethics Committee of Anhui Medical University. The TNF-α, IFN-γ, and IL-4 levels of the mice were measured using enzyme-linked immunosorbent assay kits (R&D, Minneapolis, MN). The LPS (*Escherichia coli* 055:B5) was purchased from Sigma-Aldrich (St Louis, MO). Sodium chloride injection (0.9%) was purchased from Limin pharmaceutical Co, Ltd. (Jinan, China). All other reagents were of analytical grade and were prepared by the investigators. Automatic biochemical analyzer (C16000; Abbott, Tochigi, Japan), mild hypothermia therapy apparatus (YYT-1 ice blanket machine; Chinese People's Liberation Army 6904 Factory, Taiyuan, China), and newborn's incubator (YP-920 medical infant incubator; Daiwei, Ningbo, China) were kindly provided by the Anhui Province Children's Hospital; TH212 mice thermometer (Haichuang High-Tech Science and Technology Co, LTD, Beijing, China) was provided by the Comprehensive Laboratory of Anhui Medical University, China.

2.2. Study design and regulation and management of the anal temperature of the mice

All mice had free access to drinking water and food. The mice were acclimated for 1 week at a room temperature of 22°C to 24°C, humidity of 40% to 60%, and 12-hour light/12-hour dark cycle. The study consisted of 2 parts: the first part was designed to observe the mortality rate of the mice, and the second part was designed to measure the included parameters. Different mice were used in the first and the second parts of the study.

In the first part of the study, BALB/c mice (n = 160) were divided into 2 groups, namely, sepsis group (n = 145) and normal control (NC) group (n = 15). Intraperitoneal injection of LPS (10 mg/kg) was administered for all the mice in the sepsis group, and then the anal temperature of the mice was measured 1 hour later. The mice were further divided into accompanying hypothermia group (anal temperature ≤36°C) and nonhypothermia group (anal temperature >36°C). In the accompanying hypothermia group, mice with the anal temperature lower than 34°C were excluded, and the mice with the anal temperature of 34°C to 36°C were included, which were further randomly divided into ACMH group and KN group. No thermic intervention was performed for the mice in the ACMH group, whereas the mice in the KN group were placed in the incubator to maintain the anal temperature at 36.0°C to 37.5°C. The mice in the nonhypothermia group were further randomly divided into UH group and ATMH group. No thermic intervention was performed for the mice in the UH group, whereas the mice in the ATMH group were placed in the ice blanket to maintain the anal temperature at 34°C to 36°C. The temperature of the mice in the ATMH and KN groups was tweaked to change 0.5°C/h to 1°C/h. Intraperitoneal injection of same volume of normal saline was administered for the mice in the NC group. For the mice in all the groups, appropriate food and clean water were provided. Thermic intervention lasted for 5 continuous hours. The mental state, reactions to stimulations, mobility, hair, bilateral eyes, stool, and mortality rate within 24 hours after the injection of LPS were recorded.

The processes in the second part of the study were identical to the processes in the first part. Eighteen mice from each group were selected for the examinations.

2.3. Collection and examination of the blood samples

The time at the end of the LPS injection was recorded as 0 hour. Six mice each were selected from every group at 6, 12, and 24 hours, respectively. The mice were anesthetized with ether, and then the eyeballs of the mice were removed rapidly to collect 1.0 mL of blood. The mice were then euthanized by cervical dislocation, whereas the collected blood was processed to separate serum. The serum levels of TNF-α, IFN-γ, and IL-4 were measured using enzyme-linked immunosorbent assay kits, according to the manufacturer's instructions. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA), blood urea nitrogen (BUN), and lactate were measured by the automatic biochemical analyzer (Abbott Laboratories; provided by the Laboratory Department of the Anhui Province Children's Hospital, China).

2.4. Hematoxylin and eosin staining and scoring of the lung, liver, and renal tissues

Left lung, right lobe of the liver, and the right kidney of the mice were chosen at a time point of 24 hours. The tissues were fixed with 40 g/L of paraformaldehyde, and then rinsed, dehydrated, and sliced. The slices were stained with hematoxylin and eosin, and then the pathological examination was performed with an optic microscope. Four slices were selected for each mouse for the double-blinded evaluation, and the mean scores were calculated. Acute lung injury scoring was performed by a board-certified pathologist masked to treatment assignment and was classified into 4 categories based on the severity of alveolar congestion and hemorrhage, infiltration of neutrophils in the air spaces or vessel walls, and the thickness of the alveolar wall/hyaline membrane formation [9]. The severity of each category was graded from 0 (minimal) to 4 (maximal), and the total score was calculated by summing the individual scores. The score of each animal was calculated as the mean of 4 sections.

Acute liver injury scoring was measured with the following morphologic criteria [24]: spotty necrosis, capsular inflammation, portal inflammation, ballooning degeneration, and steatosis. Spotty necrosis was graded and scored as follows: 0, none; 1, 1 focus or less per ×10 objective; 2, 2 to 4 foci per ×10 objective; 3, 5 to 10 foci per ×10 objective; and 4, more than 10 foci per ×10 objective. Capsular inflammation was graded and scored in each 10× area, after magnification, for the presence of capsular inflammation as follows: 0, none; 1, capsular inflammation in a 1 × 10 magnification area; 2, capsular inflammation in a 2 × 10 magnification area; and 3, capsular inflammation in a 3 × 10 magnification area. Portal inflammation was scored as follows: 0, none; 1, mild, some, or all portal areas; 2, moderate, some, or all portal areas; and 3, marked, all portal areas. Ballooning degeneration was scored as follows: 0, none; 1, ballooning degeneration in one third of the hepatic lobule; 2, ballooning degeneration in two-thirds of the hepatic lobule; and 3, ballooning degeneration in all parts of the hepatic lobule. Steatosis was scored as follows: 0, none; 1, <30% of hepatocytes contain fat; 2, 30%-70% of hepatocytes contain fat; and 3, >70% of hepatocytes contain fat. The liver injury severity score ranged from 0 (none) to 16 (severe).Acute kidney injury scoring was measured with the following morphologic criteria [25]: kidney damage was scored by grading glomerular, tubular, and interstitial changes. Glomerular damage (sclerotic changes such as matrix expansion, the narrowing or disappearance of Bowman space, the adhesion of the capillary tuft to Bowman capsule, the capillary collapse, and the thickening of the glomerular basement membrane) was evaluated as follows: 0, absent; 1, <25% of glomeruli affected; 2, 25%-50% glomeruli affected; and 3, >50% of glomeruli affected. The grading for tubular changes (intracellular vacuolization) was scaled as follows: 0, absent; 1, <25% of tubules injured; 2, 25% to 50% of tubules injured; and