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Therapeutic effects of compound hypertonic saline on rats with sepsis

Fang Dong[a,*], *Wei Chen*[b], *Liang Xu*[a], *Huabing Wang*[a], *Huizhi Lu*[a]

[a] *The Third Hospital of Wuhan City, Wuhan, China*
[b] *The Central Hospital of Wuhan, Wuhan, China*

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ABSTRACT

Sepsis is one of the major causes of death and is the biggest obstacle preventing improvement of the success rate in curing critical illnesses. Currently, isotonic solutions are used in fluid resuscitation technique. Several studies have shown that hypertonic saline applied in hemorrhagic shock can rapidly increase the plasma osmotic pressure, facilitate the rapid return of interstitial fluid into the blood vessels, and restore the effective circulating blood volume. Here, we established a rat model of sepsis by using the cecal ligation and puncture approach. We found that intravenous injection of hypertonic saline dextran (7.5% NaCl/6% dextran) after cecal ligation and puncture can improve circulatory failure at the onset of sepsis. We found that the levels of tumor necrosis factor-α, interleukin-1β, interleukin-6 and intracellular adhesion molecule 1 levels in the lung tissue of cecal ligation and puncture rats treated with hypertonic saline dextran were significantly lower than the corresponding levels in the control group. We inferred that hypertonic saline dextran has a positive immunoregulatory effect and inhibits the overexpression of the inflammatory response in the treatment of sepsis. The percentage of neutrophils, lung myeloperoxidase activity, wet to dry weight ratio of lung tissues, histopathological changes in lung tissues, and indicators of arterial blood gas analysis was significantly better in the hypertonic saline dextran-treated group than in the other groups in this study. Hypertonic saline dextran-treated rats had significantly improved survival rates at 9 and 18 h compared to the control group. Our results suggest that hypertonic saline dextran plays a protective role in acute lung injury caused after cecal ligation and puncture. In conclusion, hypertonic/hyperoncotic solutions have beneficial therapeutic effects in the treatment of an animal model of sepsis.

Infection and sepsis cause a very high disease rate and morbidity in patients with critical illness. Clinical epidemiological data have revealed that sepsis is one of the major causes of death in critical patients and has become the biggest obstacle in the success rate for curing high-risk diseases.[1] Obtaining a better understanding of severe infectious complications and their prevention and treatment are undoubtedly of considerable theoretical value and clinical significance. The

* *Corresponding author at*: Intensive Care Unit, The Third Hospital of Wuhan City, Wuhan, China.
E-mail address: cw99990@hotmail.com (F. Dong).
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pathogenesis of sepsis is very complicated. It is difficult to treat, and involves a series of basic problems such as infection, inflammation, immunity, blood coagulation, and tissue injury; furthermore, it is associated with pathophysiological changes in multiple body systems and organs.

Recent studies have made considerable progress on the onset and clinical significance of sepsis. However, no results have shed light on the anti-inflammatory treatment of sepsis and multi-organ dysfunction (MODS) syndrome. Currently, the standard fluid used in fluid resuscitation is an isotonic solution such as 0.9% NaCl solution and lactated Ringer's solution. Many studies have shown that hypertonic/hyperoncotic solutions used for fluid resuscitation can rapidly increase the plasma osmotic pressure, enable the interstitial fluid to return to the blood vessels rapidly and thereby restore the effective circulating blood volume,[2,3] and provide therapeutic effects for pulmonary edema caused by hemorrhagic shock.[4] Zallen et al.[5] reported that hypertonic saline can improve hemodynamic disorders in hemorrhagic shock, prevent neutrophil activation, and inhibit ICAM-1 expression by migrating through the intestinal lymphatic pathway. Junger et al.[6] found that hypertonic saline used for resuscitation in hemorrhagic shock can inhibit neutrophil and endothelial cell activation. These studies suggest that hypertonic saline can function in immune regulation during resuscitation from shock. However, doubts remain about its usefulness for the treatment of sepsis. In the present study, we developed a rat model of sepsis using the cecal ligation and puncture (CLP) approach to investigate the influence of hypertonic/hyperoncotic solutions on mean arterial pressure (MAP) and heart rate, their potential role in the regulation of serum inflammatory cytokines, and their influence on lung tissue apoptosis and tissue ICAM-1 expression in septic rats and to discuss their potential therapeutic effects in the treatment of sepsis.

Materials and methods

Establishment of a rat sepsis model

In total, 128 male Wistar rats (10–12 weeks old, weight: 230–280 g) were provided by the Institute of Zoology, Chinese Academy of Sciences. The rats were anaesthetized with intraperitoneal injection of 3% sodium pentobarbital (30–50 mg/kg). The MAP was monitored by placing a catheter in separated right common carotid artery and measured by connecting a pressure transducer. Arterial blood was sampled by placing a catheter in the carotid artery. HSD and 0.9% NaCl solution (NS) were administrated by placing a catheter in the separated left jugular vein. A rat sepsis model was established using the CLP approach. We performed traditional disinfection of the abdomen and a median skin incision of approximately 2–3 cm to expose the abdominal cavity. The cecum was freed from the mesentery; the base of the cecum was ligated with a 3-0 suture, and punctured at two sites 3 mm apart by using a No. 9 needle. The intestinal canal was then restored to ensure smooth intestinal passage, the abdominal layers were sutured, the lost fluid was replenished, and the wounds were bandaged after disinfection with tamed iodine. Operation procedures for the sham operative (SOP) group were the same as-mentioned above, except for the CLP approach.

Rat groups

The rats were randomly divided into four groups: the SOP group ($n = 21$) received a subcutaneous injection of 30 mL/kg 0.9% NaCl after the operation; the CLP group ($n = 45$) received a subcutaneous injection of 30 mL/kg 0.9% NaCl after the operation; the CLP + NS group ($n = 45$) received a subcutaneous injection of 30 mL/kg 0.9% NaCl and a jugular vein infusion of 5 mL/kg 0.9% NaCl at 0.4 mL/(kg min) for 3 h after the operation; and the CLP + HSD ($n = 28$) group received subcutaneous injection of 30 mL/kg 0.9% NaCl and a jugular vein infusion of 5 mL/kg 7.5% NaCl/6% dextran at 0.4 mL/(kg min) for 3 h after the operation.

The experiments were performed in adherence with the National Institutes of Health guidelines for the treatment of animals and ethical animal research. In addition, animals were killed at 0, 9, and 18 h after the operation. Blood samples and lung tissues were collected for examination. Equal volumes of 0.9% NaCl were immediately injected upon each blood sampling.

Determination of serum inflammatory cytokine levels

Plasma TNF-α, IL-1β, and IL-6 levels were determined using enzyme-linked immunosorbent assay (ELISA). Blood samples were collected from the common carotid artery at 0, 9, and 18 h after CLP. Blood aliquots of 0.8 mL were added to Eppendorf tubes containing sterile pyrogen-free ethylenediaminetetraacetic acid (EDTA) anticoagulant and centrifuged (3000 rpm) for 15 min at 4 °C. The plasma samples (100 μL) obtained at 0, 9, and 18 h after the operation were used, and the levels of TNF-α, IL-1β, and IL-6 were measured in duplicate using an ELISA kit (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions.

Arterial blood gas analysis

The carotid artery blood of rats in each group was sampled (0.2 mL) at 0, 9, and 18 h after the operation, and the pH, PaO_2, and $PaCO_2$ in the blood samples were assayed using a portable blood gas analyzer (i-STAT, Abbott, Chicago, USA).

Percentage of bronchoalveolar lavage fluid (BALF) neutrophils

BALF collection and cell sorting methods were performed as reported by Reis et al.[7] and Callol et al.[8] The trachea and lung of the sacrificed rats were exposed at 18 h after the operation. The right bronchus was ligated, the trachea was fixed on the annular cartilage with forceps, and a transverse incision was made under it. By removing the No. 7 needle, an approximately 10-cm-long plastic infusion tube was kept remaining. The tube was inserted into the tracheal cavity, sutured at one end, and fixed on a 5-mL syringe at the other end. Sterile saline (2 mL, 37 °C) was slowly injected into the lung, and suctioned back after 30 s. The above procedures were repeated thrice and a total of 5 mL of BALF was collected. The BALF was