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Comparison of two different models of sepsis induced by cecal ligation and puncture in rats

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ABSTRACT

Background: The present study was designed to explore the difference between two rat models of sepsis and to establish a more stable rat model.

Materials and methods: Sprague–Dawley rats were randomly divided into three groups: sham operation group, simple cecal ligation and perforation group (SCLP), and cecal ligation perforation plus drainage group (CLP-DS). The general condition of the rats was observed, and the time of death and survival rate at 72 h were recorded. The arterial blood and lung tissue were obtained 9 h after the operation.

Results: The mortality of the CLP-DS group was significantly higher than that of the SCLP group. The limitation package, intestinal adhesion, and poor drainage were detected in the SCLP rats, whereas intestinal edema and hyperemia, bloody water in the abdominal cavity, but no inflammatory package were observed 24 h after the operation in the CLP-DS rats by autopsy. There were significant differences in interleukin-6 and tumor necrosis factor-alpha levels between the SCLP group and the CLP-DS group. Severe pulmonary septal thickening, alveolar wall vascular congestion, and protein debris deposition in the alveolar cavity were observed in the SCLP group, whereas pulmonary bullae were observed in the CLP-DS group using light microscopy, and there were significant difference among groups in Smith lung injury score.

Conclusions: These results suggested that the cecal ligation combined with puncture drainage model of sepsis is more stable than that of the simple cecal ligation and puncture model of sepsis in the rat, which resolved the problem of puncture wrapped in the traditional CLP model of sepsis in rat.

Introduction

Sepsis is increasingly common in clinical practice and has extremely high mortality; it is a major complication of serious trauma, burn, shock, and major surgery and is also one of the leading causes of death in critically ill patients.[1-3] The cecal ligation perforation sepsis model is most similar to clinical sepsis, and a rat or mouse cecal ligation perforation (CLP) is commonly used to mimic clinical appendicitis perforation or diverticulitis perforation, which has been recognized as the gold standard of sepsis animal modeling.[4,5] However, the morbidity and mortality are unstable in the cecal ligation puncture sepsis model due to many factors, which makes these experimental studies

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challenging. In this study, we compared the rat cecal ligation perforation sepsis model with ligation perforation and the drainage sepsis model to generate a sepsis research model with more stable morbidity and mortality.

Materials and methods

Experimental animal and group

The experimental protocol was approved by the Ministry of Science and Technology Committee and Animal Center Committee and Logistics University of Chinese People Armed Police Force Hospital. Animal experiments adhered to the ethical guidelines of the International Association for the Study of Pain, and all efforts were made to minimize animal suffering and to reduce the number of animals used. The experiments in this study were performed on male, Sprague–Dawley rats (260 g-300 g) housed under approved conditions with a 12-/12-h light/dark cycle and food and water provided ad libitum. The experiments were performed at the same time period on different days to reduce the effect of circadian rhythms on different individuals. The animals were randomly divided into three groups: sham operation group (sham) ($n = 16$), simple cecal ligation perforation group (SCLP) ($n = 32$), and cecal ligation perforation plus drainage group (CLP-DS) ($n = 32$). Only open abdominal cavity operation was performed on rats in the sham group. After the experiments, the rats were euthanized via decapitation under deep anesthesia by ketamine (intraperitoneal injection).

Establishment of rat model

SCLP model

The rats were anesthetized by 1% ketamine (100 mg/kg) via an intraperitoneal injection after weighing and then fixed on the small animal operating platform board. After abdominal skin preparation and disinfection, a longitudinal incision (length 2 ~ 2.5 cm) was performed along the ventral line above the pubic bone to open the celiac. The cecum was identified, and it was then carefully taken out. The contents of the cecum were gently pushed to the cecal distal to reduce the gas of ligation cecum, and the mesentery was carefully separated to avoid injury of the ileocecal artery cecal branch. The free distal cecum was placed in methylene blue solution in a sterile cylinder until a clear blue mark was observed around the cecum, at a volume of 2.5 mL mark. The cecum was ligated with 4-0 silk thread according to the mark. Intestinal obstruction did not occur because the ligation sites were lower than the location of the ileocecal valve. Next, an 18 G (1.2 mm × 3.8 mm) needle was applied to take a single pass through the cecum from the mesenteric side to the contralateral side. After the puncture, a small amount of feces was squeezed from the two puncture holes to ensure perforation (supplemental Fig. 1). The cecum was placed back into the abdominal cavity after the feces out of the cecum was wiped off. Next, the muscle and skin were sutured with a silk thread (3-0) to close the abdomen. Preheated saline (50 mL/kg, 37°C) was subcutaneously injected into the back for resuscitation. The rats were placed back into the cage after surgery, and food and water were provided ad libitum.

CLP-DS model

The specific steps were performed as follows: the preoperative preparation and abdominal cavity opening were performed similar to the SCLP model. After cecum ligation and puncture, the two puncture holes were kept opened with a drainage bar across (twisted strip of sterile gauze with a width of 2 mm) to make sure that the strip was exposed 5 mm outside the two holes. The remaining length was cut off, and the feces around the area were wiped off (Supplemental Fig. 1).

Indicators and testing methods

- General postoperative observation, including the appearance, activities, mental state of the rats, among other factors, was performed.
- The death time point and survival rate of the rats were recorded at 72 h after modeling, and the changes in abdominal cavity were observed 24 h after modeling.
- Six rats of each group were randomly selected, and 3 mL abdominal aorta blood was obtained 9 h after modeling and placed in an anticoagulant tube for 30 min at 4°C. Next, the samples were centrifuged for 15 min at 3000 r/min, and 200 μL serum was placed in a cryopreserved tube for cryopreservation at −80°C. The serum interleukin (IL)-6 and tumor necrosis factor (TNF)-α levels were subsequently detected using an enzyme-linked immunosorbent assay.
- Lung tissue observation: The lung tissue was fixed with 4% neutral formaldehyde solution for 48 h, then dehydrated, paraffin-embedded, sliced (5 μm), baked, and HE stained. The histopathological changes were observed under a light microscope, and the lung injury was evaluated by Smith lung injury score. According to the Smith score, the pulmonary edema, alveolar and interstitial inflammation, alveolar and interstitial hemorrhage, pulmonary atelectasis, and hyaline membrane formation were semiquantitative analyzed from 0 to 4 point: noninjury was 0 point, the lesion less than 25% of visual field was 1 point, the lesion from 25% to 50% of visual field was 2 point, the lesion from 50% to 75% of visual field was 3 point, and the lesion in full view was 4 point; the total lung injury score was the sum of the aforementioned. Ten visual fields of each rat were observed, and the average value was adopted.

Statistical analysis

The data were expressed as the mean ± standard deviation ($\overline{X} \pm s$), and statistically analyzed using SPSS 17.0 software. The survival rate of each group was analyzed using Kaplan–Meier method (log-rank test). IL-6, TNF-α levels, and Smith lung injury scores among groups were analyzed by single factor analysis of variance. A P-value less than 0.05 was considered statistically different.

Results

General condition of the rats after modeling

The experimental rats were awakened from anesthesia 1-2 h after operation. The rats in the SCLP and CLP-DS groups