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Survival advantage depends on cecal volume rather than cecal length in a mouse model of cecal ligation and puncture

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ABSTRACT

Background: Cecal ligation and puncture (CLP) is the most commonly used model to simulate human polymicrobial sepsis. However, the severity of CLP is difficult to be standardized across different laboratories. The aim of the present study was to evaluate the influence of ligated cecal volume and length on mortality in mouse CLP model.

Methods: Cecal length and volume were measured from 120 Kunming mice subjected to CLP or sham operation. According to cecal volume, mice were divided into three groups, volume$_{0.0\sim0.2}$ (0.0 cm^3-0.2 cm^3), volume$_{0.2\sim0.4}$ (0.2 cm^3-0.4 cm^3), and volume$_{>0.4}$ (larger than 0.4 cm^3). The contents of cytokines, including interleukin-1β, interleukin-6, and TNF-α, were measured at 3 h after surgery. The blood bacterial load and oxidative stress indicators (including malondialdehyde and superoxide dismutase) were measured at 12 h after surgery.

Results: There was no significant difference on 72-h survival rate between the mice with cecum longer than 2 cm and shorter than 2 cm. Compared to the other volume groups, volume$_{>0.4}$ group showed significantly increased blood bacterial load, malondialdehyde levels in lung and liver, and pro-inflammatory cytokines in serum. Surprisingly, the survival rate in volume$_{>0.4}$ (0%) group showed significant difference from those of volume$_{0.0\sim0.2}$ group (40%) and volume$_{0.2\sim0.4}$ group (40%).

Conclusions: The mice in volume$_{>0.4}$ group have much serious inflammatory reaction and are easier to die. As the proportion of volume$_{>0.4}$ mice is near 20%, it can have large influence on most of the related studies using this CLP model.

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Introduction

Sepsis is an excessive systemic host response to the invasion of pathogenic microorganisms or toxins. Despite more use of specific antibiotics, surgical intervention, invasive monitoring, volume substitution, and organ replacement, sepsis is still the most common cause of death in the intensive care unit, which can result in about 30%-50% of patients with excessive tissue damage, and even death.[1] The invasion of pathogens such as bacteria, viruses, fungi, and parasites is the most common factor inducing sepsis. The spread of microbes into the blood and tissues can trigger an inflammatory response that releases many cytokines and chemokines throughout the body, which ultimately causes negative effects on multiple organs.[2] In past decades, several experimental models have been developed to simulate clinical sepsis, including lipopolysaccharide injection, colon ascendens stent peritonitis, cecal ligation and puncture (CLP) and intratracheal and abdominal administration of microbial pathogens.[3-6]

CLP model is invented by Chaudry *et al.* in 1979.[7] It is a most commonly used procedure to simulate human sepsis because it can closely recapitulate the pathophysiological responses observed in human septic patients with the infectious nidus originally located in the abdominal compartment.[2] However, despite the clinical relevance and widespread use of this model, it also has some shortcomings. One of the major shortcomings is the variability of mortality observed by different laboratories and even different experiments. The major determinants of mortality include the size of the needle used to make cecal puncture, the number of punctures in cecum (usually ranging from one to four), the utilization of antibiotics and/or fluid resuscitation, and animal gender.[8-10] However, in 2003, K.D.Singleton *et al.* discovered that significant differences still exist in the CLP models with similar needle size, puncture number, and fluid resuscitation. In addition, their data demonstrate that the length of ligated cecum is also a major determinant of mortality in the CLP model of sepsis. It may be because longer cecum can store more stools, which leads more pathogens to inoculate into blood.[9]

However, in our previous study, we discover that cecal shape is irregular and variable. Cecal length can be easily changed especially during the through-and-through puncture and extrusion. Therefore, cecal volume rather than length may be a more precise indicator of the amounts of stool, and it may stay the same during surgery. Therefore, the aim of this study was to evaluate the effectiveness of cecal volume as a determinant of mortality.

Methods

Animals and model selection

All animal experiments in this article were performed according to the Guide for the Care and Use of Laboratory Animals of Sun Yat-sen University (permit numbers: SCXK (Guangdong) 2009-0011). A total of 120 Kunming male mice (7-8 wk and 25-27 g) were purchased from the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China) and housed in a temperature and light-cycle controlled room in animal facilities, with unlimited food and water.

A nearly 80% mortality was reached at 72 h after surgery in 72-h CLP model. Our laboratory aims to study human acute and severe sepsis. This model could induce severe inflammatory reaction, which could meet the experimental requirements. In addition, we have practiced and repeated it for hundreds of times in our previous study.[11] We could control intergroup variability to a low level. However, we could not ensure the same proficiency and standard survival rate in other CLP techniques.

Experimental protocol

Animals were fasted for 6 h before CLP surgery. After the anesthesia by intraperitoneal injection of chloral hydrate (10%), a 1.5-cm incision was made in the abdominal wall, and cecum was extruded carefully. Cecum was then ligated just below the ileocecal valve with a 4-0 silk suture (Jinhuan Medical, Shanghai, China) to avoid bowel obstruction. Cecal length was measured from the tip of ascending cecum to the tip of descending cecum as described by Singleton and Wischmeyer.[9] We measured cecal volume directly after ligating the cecum. We turned the mouse upside down and pulled the ligated parts to a sterile measuring cylinder containing 2-mL sterile saline solution. The increase of scale was recorded and served as the volume of the ligated cecum. After measurement, we used a 21-G needle to perforate cecum by single through-and-through puncture midway between the ligation and the tip of cecum in the mesenteric-to-antimesenteric direction (into the cecum on one side and through the cecal wall on the opposite side).[8] After removing the needle, we extruded a small amount of feces from both the mesenteric and antimesenteric penetration holes to ensure patency. In the sham group, mice abdomen was opened, and mice cecum received the same manipulations, but no CLP was performed. Resuscitate animals by subcutaneously injecting normal saline (37°C; 5 mL per 100 g body weight).

To minimize intergroup variability and increase efficiency, all the CLP surgeries were performed by T.S., and the measurements of cecal length and volume were performed by H.Y.

Survival rate

Twenty mice were randomly selected from a total of 120 mice to serve as the sham group. Half of them were randomly selected to obtain the 72-h survival data, and the rest were used to collect specimens. Fifty mice were randomly selected from the rest 100 mice and were used to obtain the 72-h survival data after CLP surgery. All mice had free access to water and food and were monitored at 6, 12, 24, 48, and 72 h after surgery. No other experiment was performed on the mice for collecting survival data.

Specimen collection

The rest 50 mice were used to collect specimens. To measure serum cytokines, blood samples were collected through the