Efficacy of Shenfu decoction on sepsis in rats with condition induced by cecal ligation and puncture

Liu Jin, Liu Fusheng, Liang Tengxiao, Cao Po, Li Jing, Zhang Yin, Liu Yang

METHODS: Forty clean-grade male Sprague-Dawley rats were divided randomly into three groups: normal control group (NCG, n = 15), model control group (MCG, n = 15) and Shenfu decoction group (SFDG, n = 15). Sham-operated rats in NCG were served as operation control, while rats in both MCG and SFDG were exposed to CLP, a procedure to develop experimental sepsis. Rats in SFDG were administered with SFD by gavage (3 mg/g of body weight, twice a day) 2 h prior to CLP and directly after successful CLP, while rats in NCG and MCG were gavaged with equivalent volume of sterilized water. Rats in all groups were starved with free access to drink. After 24 h of administration, the mortality of rats in each group was assessed. The indicators of inflammatory response [the peritoneal inflammation by Simon’s method Classification as well as serum concentrations of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) by enzyme linked immunosorbent assay (ELISA)] in survival rats were evaluated. The indicators of gut barrier [The intestinal mucosal injury index, serum concentrations of D-lactic acid and secretory IgA (sIgA) in intestinal mucosa by ELISA, as well as gut microbiota by 16S rRNA gene sequencing] in survival rats were evaluated.

RESULTS: The mortality (20%) of rats in SFDG was lower than that (33.3%) of the MCG (P < 0.01). The mortality (20%) of rats in SFDG was lower than that (33.3%) of the MCG (χ² = 6.533, P = 0.011). Compared with the MCG, the peritoneal inflammation as well as serum concentrations of TNF-α and IL-6 decreased significantly in SFDG (all P < 0.01). Compared with the MCG, the IMII, serum concentrations of D-lactic acid, sIgA in intestinal mucosa were alleviated by SFD treatment (all P < 0.01). Increase in

Abstract

OBJECTIVE: To evaluate the efficacy of Shenfu decoction (SFD) prepared with a traditional Chinese formula, on sepsis in rats with the condition induced by cecal ligation and puncture (CLP), and to study the possible mechanism underlying its action.
levels of Proteobacteria and reduction levels of Bacteroidetes induced by sepsis were observed, and these two disturbed gut microbiota phyla could be regulated after SFD treatment. Increase in levels of Proteobacteria and reduction levels of Bacteroidetes induced by sepsis were observed, and these two disturbed gut microbiota phyla could be regulated after SFD treatment.

**CONCLUSION:** SFD may play a protective role in sepsis by alleviating sepsis-induced inflammatory response and gut barrier damage in rats.

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**Keywords:** Sepsis; Punctures; Inflammation; Shenfu decoction; Gut barrier

**INTRODUCTION**

Sepsis is defined as life-threatening organ dysfunction due to dysregulated host reactions to infection. As sepsis worsens, septic shock may develop, which is potentially fatal and has been associated with a greater risk of mortality than sepsis alone. The "sepsis bundle" refers to a series of emergent responses of medical implementation to septic shock, and has been regarded as cornerstone of sepsis quality improvement since 2005. The current bundle treatment of sepsis involves fluid resuscitation, appropriate antibiotics, administration of vasoactive agents, ventilatory support, and blood purification. Despite waning evidence supporting these bundles, the dramatically increased incidence of sepsis and septic shock suggests that this cornerstone is facing retirement. Traditional Chinese formulae have long been applied to combat infectious diseases. Based on TCM theory, the acute asthenia syndrome, a psychopathological condition characterized by extreme acute loss of strength, is an important element of sepsis and is highly relevant to the prognosis of sepsis. Shenfu decoction, a well-known Traditional Chinese herb-couple formulation, functions to revive Yang Qi and prevent collapse, as occurred in acute asthenia syndrome. Shenfu injection, a processed form of Shenfu decoction, has been widely used to integrate with routine treatments and accepted as a part of therapies for sepsis shock in Chinese clinical practices to improve physical conditions of patients. Mechanisms of actions of Shenfu injection have been previously proposed to reduce inflammatory responses, improve cellular immune function and alleviate intestine epithelial damages. However till now, no direct and detailed studies investigating therapeutic effects and mode of actions of Shenfu decoction on sepsis have been conducted. In this study, we aimed to investigate the efficacy of Shenfu decoction prepare with a traditional Chinese formula, against sepsis in rats with the condition induced by cecal ligation and puncture (CLP), and to study the mechanism behind the action.

**METHODS**

**Preparation of Shenfu decoction**

Shenfu decoction is prepared with: 30 g Renshen (Radix Ginseng) and 15 g Fuzi (Radix Aconiti Lateralis Preparation), which were purchased from Beijing Tcmages Pharmaceutical Co., Ltd. (Beijing, China) Quality control of Shenfu decoction was performed by infrared fingerprint. Shenfu decoction was resuspended in sterilized water at a final concentration of 1 g/mL.

**Animals**

Forty clean-grade male Sprague-Dawley rats (3 months old, 200-240 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China, Production license: Beijing-2016-0011) and raised in the animal house of Beijing University of Chinese Medicine Dong Fang Hospital. Prior to experiments, all animals were acclimatized under controlled humidity (60% ± 5%), temperature [(20 ± 2 ℃) and 12 h light/12 h dark cycle for 3 d.

**Rat sepsis model induced by CLP**

All animal experiments in the study were approved by the Administrative Committee of Experimental Animal Care and Use of Beijing University of Chinese Medicine Dong Fang Hospital. All experimental procedures were conformed to the National Institute of Health guidelines on the ethical use of animals. To replicate the rat sepsis model, the CLP procedure was performed according to that described previously. Briefly, rats were anesthetized using 10% chloral hydrate (0.4 mL/100 g, i.p.). After preparing skin and disinfecting the abdominal area by iodine, a midline laparotomy was made using minimal dissection and the cecum was exposed and ligated at the distal end of the cecum with a sterile 4 silk thread binding tightly, so that intestinal continuity was maintained. The antimesenteric surface of the cecum was perforated with an 18-gauge needle at two locations 1 cm apart and the cecum was gently compressed until fecal matter was extruded. The bowel was then returned to the abdomen and the incision was closed. Rats in all groups were returned to their cages and starved with free access to drink.

**Experimental procedures**

With a random number table, forty clean-grade male Sprague-Dawley rats were randomly divided into normal control group (NCG, n = 10), model control group (MCG, n = 15), and Shenfu decoction group (SFDG, n = 15). Rats in NCG were subjected to sham operation that was given a laparotomy, and the cecum was manipulated but not ligated or perforated. The
rats of MCG and SFDG were used to replicate the rat sepsis model using CLP. Rats in SFDG were administered with SFD by gavage (3 mg/g of body weight, twice a day) both 2 h prior to CLP and after successful CLP, while rats in NCG and MCG by gavage of equivalent volumes of sterilized water. Feces samples were collected both before and after treatment of each group by forcing to defecate. After 24 h, the rats were anesthetized with an intraperitoneal injection of 10% hydrochloric acid. A midline laparotomy was made for assessing peritoneal inflammation. The peripheral blood was collected via the abdominal artery using a 23-gauge needle and a 5 mL syringe by inserting the needle into the artery and drawing blood slowly. Serum was obtained after centrifugation (Eppendorf, Centrifuge 5810R; 3000 rpm for 10 min) of the samples. Then the serum samples were repackaged and at −80 °C until analysis. Serial in viv5 cm intestinal biopsies distant from the cecum were taken prior to and during the hypoperfusion period at from 5 to 30-min intervals. The intestinal specimens were cut open and the contents of the intestine were rinsed with PBS. Then half of intestinal biopsies were fixed with 10% formaldehyde solution, and the other half frozen at −80 °C until analysis.

**Mortality and peritoneal inflammation assessment**

After 24 h of administration, the mortality of rats in each group was assessed. Peritoneal inflammation was evaluated according to Simon’s method Classification.

**Histopathological assessment assessed by intestinal mucosal injury index (IMII)**

For histopathological examination, formalin fixed, paraffin-embedded intestine tissues were cut into serial sections (5 µm thick) and stained with hematoxylin and eosin (HE). Microscope (OLYMPUS U-CMAD-2) and image acquisition system (Nikon DS-Fi2) were used for histological Image acquisition. Histological alterations were assessed by IMII based on the criteria of the specific mucosal changes graded as follows:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Grade criteria of peritoneal inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No inflammation</td>
</tr>
<tr>
<td>1</td>
<td>Well-walled-off abscess, no free peritoneal fluid</td>
</tr>
<tr>
<td>2</td>
<td>Walled-off abscess, small amount of free peritoneal fluid, and patchy areas of cecal wall necrosis</td>
</tr>
<tr>
<td>3</td>
<td>Poor localization of the inflammatory process, moderate amount of free peritoneal fluid, and gangrenous cecum</td>
</tr>
<tr>
<td>4</td>
<td>No walling off the cecum, large amount of free hemorrhagic fluid in the peritoneal cavity, and extensive gangrene and hemorrhagic fluid in the cecal wall</td>
</tr>
</tbody>
</table>

**Measurement of D-lactic acid, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in peripheral blood and secretory IgA (sIgA) in intestinal mucosa**

The concentrations of D-lactic acid, TNF-α and IL-6 in peripheral blood were detected by ELISA kit (Abcam, Shanghai, China) according to the instruction strictly. The intestinal specimens were cleared at liquid nitrogen and then extracted by RIPA neutral lyase and ultrasonic. The homogenate of intestinal specimens was centrifuged (12000 rpm, 15 min) and supernatant was collected. The concentrations of sIgA in supernatant were determined by ELISA kit (Lifespan, WA, USA, LSF28068) according to the instruction strictly. All fractionation procedures were performed under ice-cold conditions. Micropipette and enzyme marker (Thermo Scientific, Wilmington, USA) were used in test.

**Fecal sampling and 16S rRNA gut microbial community analysis**

Before and after treatment, feces samples were collected form rats of NCG, MCG and SFDG. At least 2 pellets of feces were collected from each rat, transferred into sterile conical tubes, immediately frozen in liquid nitrogen and stored at −80 °C for microbiological detected using 16S rRNA gene sequencing. The feces of mice were sent to Shanghai Meiji Biomedical Technology Co., Ltd. for 16S rRNA analysising. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%.

<table>
<thead>
<tr>
<th>Score</th>
<th>Pathological score criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal mucosal villi</td>
</tr>
<tr>
<td>1</td>
<td>Development of subepithelial Gruenhagen’s space, usually at the apex of the villus; often with capillary congestion</td>
</tr>
<tr>
<td>2</td>
<td>Extension of the subepithelial space with moderate lifting of epithelial layer from the lamina propria</td>
</tr>
<tr>
<td>3</td>
<td>Massive epithelial lifting down the sides of villi. A few tips may be denuded</td>
</tr>
<tr>
<td>4</td>
<td>Denuded villi with lamina propria and dilated capillaries exposed posed. Increased cellularity of lamina propria may be noted</td>
</tr>
<tr>
<td>5</td>
<td>Digestion and disintegration of lamina propria; hemorrhage and ulceration</td>
</tr>
</tbody>
</table>
Statistical analysis
The continuous results were presented as mean ± standard deviation (x ± s). Data were processed with SPSS 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA). One-way analysis of variance (ANOVA), t-test, Mann-Whitney U test, and χ² test were conducted to test the differences between groups. P < 0.05 was the statistically significant level.

RESULTS
SFD reduced mortality and peritoneal inflammation
The sepsis model was successfully established 12 h after operation comparison with heat rate (HR), body temperature (BT) and procalcitonin (PCT) of the sham group (P < 0.05) (Table 3).

Table 3. Comparison of HR, BT, and PCT between sham and CLP groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>HR (beats/min)</th>
<th>BT (°C)</th>
<th>PCT (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCG</td>
<td>10</td>
<td>313.3±41.2</td>
<td>36.7±0.4</td>
<td>0.37±0.02</td>
</tr>
<tr>
<td>CLP</td>
<td>10</td>
<td>552.4±32.5°</td>
<td>38.8±0.2°</td>
<td>1.06±0.05°</td>
</tr>
</tbody>
</table>

Notes: rats in SFDG were administered with SFD by gavage (3 mg/g of body weight, twice a day) both 2 h prior to CLP and after successful CLP, while rats in NCG and MCG by gavage of equivalent volumes of sterilized water. HR: heat rate; BT: Body temperature; PCT: Procalcitonin; CLP: Cecal Ligation and Puncture. Data are mean ± SD. P < 0.05 vs sham group.

No death occurred in NCG, while the SFD therapy significantly reduced the mortality (20%) of rats compared with that (33.3%) of the MCG (P = 0.011) 24 h after implementation of the treatment. Rats underwent relaparotomy immediately after mortality or anesthesia for survivals 24 h after completion of the SFD therapy. Following the grading of intra-abdominal sepsis according to Simon’s method, peritoneal inflammation was evaluated. With respect to Simon’s grade, the grade of peritoneal inflammation of the rats in MCG was mainly grade 3 and grade 4, which was more severe than that of NCG, whereas the grade of peritoneal inflammation could be substantially alleviated by SFD (Figure 1).

SFD reduces intestinal mucosal injury
The intestinal mucosal tissue of the rats in NCG was intact and the intestinal villi were arranged in order under the optical microscope, whereas villi were severely damaged in MCG and SFDG (Figure 2). There was mucosal thickness thinning, local visible lamina propria disruption, villous epithelium stripping, intestinal villus arrangement, edema within the villi of the subcutaneous gap, along with neutrophil infiltration in rats of MCG and SFDG. Compared with NCG (0.26 ± 0.33), the IMII of rats in MCG (4.14 ± 0.56) and SFDG (1.72 ± 0.59) were significantly increased according to pathological scores criteria, P < 0.001. Compared with MCG, the IMII of rats in SFDG were significantly decreased P < 0.001 (Figure 3A) according to pathological scores criteria.

Laboratory parameters of DLA, TNF-α, IL-6 and sIgA
Because no blood samples could be drawn from dead rats, the laboratory parameters were evaluated over survivals in MCG and SFDG. TNF-α in peripheral blood serum of rats in the NCG < SFDG < MCG (P = 6.492, P = 0.049). IL-6 in peripheral blood serum of rats in the NCG < SFDG < MCG (F = 101.287, P = 0.000). D-lactic acid in peripheral blood serum of rats in the NCG < SFDG < MCG (F = 200.092, P = 0.000). sIgA in intestinal mucosa of rats in MCG < SFDG < NCG (F = 62.721, P = 0.000). The D-lactic acid, TNF-α and IL-6 in peripheral blood of SFDG could be substantially alleviated by SFDG treatment, while sIgA in intestinal mucosa be significantly increased by SFDG treatment compared with that of the MCG.

Figure 1 Comparison of peritoneal inflammation between groups
A: NCG; B: MCG; C: SFDG; D: Grade of peritoneal inflammation between NCG, MCG and SFDG groups by Simon’s method Classification. Grade 0 is the lightest, Grade 4 is the heaviest. Rats in SFDG were administered with SFD by gavage (3 mg/g of body weight, twice a day) both 2 h prior to CLP and after successful CLP, while rats in NCG and MCG by gavage of equivalent volumes of sterilized water. NCG: normal control group; MCG: model control group; SFDG: Shenfu decoction Group; CLP: Cecal Ligation and Puncture.
Figure 2 Comparison of intestinal mucosal injury between groups(HE staining in intestinal mucosa of survival rats, slice thickness 5 μm; original magnification ×100)
A: NCG; B: MCG; C: SFDG; D: comparison of intestinal mucosal injury index scores between groups. Rats in SFDG were administered with SFD by gavage (3 mg/g of body weight, twice a day) both 2 h prior to CLP and after successful CLP, while rats in NCG and MCG by gavage of equivalent volumes of sterilized water. HE: hematoxylin and eosin; NCG: normal control group; MCG: model control group; SFDG: Shenfu decoction group; CLP: cecal ligation and puncture. Data are shown as mean ± standard deviation. *P < 0.05, **P < 0.05, versus NCG; *P < 0.05, versus MCG; SD: Standard deviation.

Table 4 Comparison between groups of DLA, TNF-α, IL-6 and slgA

<table>
<thead>
<tr>
<th>Items</th>
<th>NCG (n = 10)</th>
<th>MCG (n = 10)</th>
<th>SFDG (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/mL)</td>
<td>79.8±46.3</td>
<td>228.5±181.3*</td>
<td>83.0±36.2*</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>130.6±12.1</td>
<td>158.4±9.5*</td>
<td>99.9±5.1*</td>
</tr>
<tr>
<td>D-lactic acid (μg/mL)</td>
<td>7.7±1.4</td>
<td>11.2±0.7*</td>
<td>8.8±0.6*</td>
</tr>
<tr>
<td>slgA (μg/mL)</td>
<td>26.3±2.0</td>
<td>11.6±0.3*</td>
<td>20.9±1.6*</td>
</tr>
</tbody>
</table>

Notes: rats in SFDG were administered with SFD by gavage (3 mg/g of body weight, twice a day) both 2 h prior to CLP and after successful CLP, while rats in NCG and MCG by gavage of equivalent volumes of sterilized water. DLA: D-lactic acid; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; slgA: secretory IgA; NCG: normal control group; MCG: model control group; SFDG: Shenfu decoction Group; CLP: Cecal Ligation and Puncture. Data are shown as mean ± standard deviation. *P < 0.05, versus NCG; **P < 0.05, versus Group MCG.

**Regulation effect of gut microbiota by SFD**
Fecal microbiota composition profiles were analyzed by 16S rRNA gene sequencing-based method. There are 1 Domain, 1 Kingdom, 13 Phylum, 21 Class, 31 Order, 53 Family, 140 Genus, 280 Species and 749 OTU. Around 99% of the total bacterial abundance was classified into fix phyla, while the rest was allocated to various unclassified bacteria. The dominant phyla including Firmicutes, Bacteroidetes and Proteobacteria (Figure 3A). There were differences of intestinal flora among the three groups at the gene level (Figure 3B, 3C). These results suggested that changes of the gut microbiota at the phylum and genus level between NDG, MCG and SFDG. CLP treatment resulted in a significant increase in levels of Proteobacteria and reduction levels of Bacteroidetes, whereas these two disturbed gut microbiota phyla could be regulated by SFD (Figure 3D). To compare community patterns, Partial Least Squares Discriminant Analysis (PLS-DA) was used. A scatter plot based on PLS-DA scores from the sequences at OUT level showed a clear separation of the community composition between NCG, MCG and SFDG (Figure 3E). The PLS-DA scores revealed that CLP group was significantly different from NCG and after administration of SFD, the overall similarity was recovered (Figure 3E). The type of intestinal flora in the MCG was lower than that in the NCG, while SFD can restore the diversity of gut microbiota comparing with the MCG (Figure 3F). These above results showed that Shen-Fu Decoction regulate gut microbiota dysbiosis induced by sepsis.

**DISCUSSION**
Sepsis increases activation of the inflammatory response and induces gut microbiota dysbiosis, along with compromised epithelial integrity.15,16 Excessive inflammation during severe sepsis may induce injured gut mucosa and cause tight junction damage in the intestinal mucosal epithelium, which enables gut microbiota dysbiosis and bacterial translocation.17 The gut has been characterized as the motor of multiple organ dysfunction syndrome in the progression of sepsis and sepsis shock.18 TNF-α, the main inflammatory mediator of sepsis, was shown to induce intestinal permeability.19 IL-6, which transiently produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase responses.20 Previous studies have shown that proinflammatory cytokines TNF-α and IL-6 contribute to the inflammatory process and cause an increase in intestinal tight junction permeability by allowing luminal antigenic penetration.21,22 Failure of the gut barrier remains central to the hypothesis that toxins escaping from the gut lumen contribute to activation of the host’s immune inflammatory defense mechanisms.23 Evidence has been showed that the increased intestinal permeability demonstrated in critical patients was associated with the progress of sepsis.24 Accumulating evidence suggests that the integrity of the gut epithelium and competence of adaptive immune responses play a critical role in the progression of sepsis and multiple organ dysfunction.
D-lactic acid is the product of gastrointestinal bacterial fermentation and cannot be degraded rapidly. The increase of permeability of intestinal tract can lead to the increase the level of D-lactate in peripheral blood, which can reflect the integrity and damage degree of the intestinal mucosa. The gut barrier consists of mechanical, immunologic, chemical, and biological components. The gut immunological barrier and gut microbiota are interrelated and interact on each other. Decreases in the level of sIgA during sepsis suggest that LPS-induced endotoxemia inhibited humoral immune function of the digestive tract.

Shenfu decoction is consisted of Renshen (Radix Ginseng) and Fuzi (Radix Aconiti Lateralis Preparata), whose main components are ginsenosides and alkaloids. Shenfu decoction has the functions of restoring Yang and saving the reverse. Shenfu decoction is clinically used for shock, heart failure, sepsis and has been

Figure 3 Regulation effect of gut microbiota by SFD
A: the gut community profiles at the phylum level among NCG, MCG and SFDG; B, C: the gut community profiles at the genus level NCG, MCG and SFDG revealed by 16S rRNA gene sequencing (each color represents one bacterial genus); D: the gut microbiota of Multi-species difference test among NCG, MCG and SFDG; E: The gut microbial population test among NCG, MCG and SFDG.
NCG: normal control group; F: the gut microbiota patterns of NCG, MCG and SFDG by PLSDA on Genus level; MCG: model control group; SFDG: Shenfu decoction Group. Fecal microbiota composition profiles of NCG, MCG, and SFDG were analyzed by 16S rRNA gene sequencing-based method.

comp1 (14.04%)
comp2 (12.48%)
-7.5
-5
-2.5
0
2.5
5
7.5
-6 -4 -2 0 2 4 6

A
B
C
D
E
F

Figure 3
come a necessary medicine for emergency rescue. Studies have shown that Shenfu decoction can regulate inflammation and immunity. To explore the effects of Shenfu decoction on sepsis, we applied CLP method, which has been proposed to closely replicate the nature and course of clinical sepsis in humans. Therefore, to further clarify the mechanisms of SFD, host inflammatory response (peritoneal inflammation as well as serum TNF-α, IL-6 and IL-10), the intestinal integrity (IMII, D-lactate in peripheral blood and sIgA in intestinal mucosa as the indicator of intestinal immunological barrier), along with gut microbiota (indicator of biological barrier) were measured in this study. Our results show that Shenfu decoction can decrease the mortality of rats exposed to sepsis. First, this study suggests that Shenfu decoction can significantly decrease peritoneal inflammation as well as the serum level of TNF-α and IL-6 induced by sepsis. Second, the intestinal integrity was evaluated by IMII, D-lactate in peripheral blood and sIgA in intestinal mucosa. CLP treatment resulted in significant increase IMII, D-lactate in peripheral blood and reduction levels of sIgA in intestinal mucosa, whereas these three disturbed indicators could be alleviated by Shenfu decoction. Thus, it is clearly presented that Shenfu decoction may play a protective role in sepsis by alleviating intestinal mechanical and immunological barrier. Previous studies have demonstrated that Shenfu injection significantly alleviates intestinal epithelial damage and ameliorates the mucosal barrier function in septic rats in a dose-dependent manner. Consistent with Shenfu injection, Shenfu decoction is also effective in ameliorating the mucosal barrier dysfunction. However, this study did not further explore the possibilities of Shenfu decoction in increasing gut cell tight junction. Third, the regulation of gut microbiota by Shenfu decoction was analyzed by 16S rRNA gene sequencing-based method. Pioneer evidence suggests that both the gut microbiota dysbiosis and the pathological symptoms can be alleviated by traditional Chinese medicines. Therefore, we provided the first evidence that Shenfu decoction could significantly increase the levels of Bacteroidetes and reduce the levels of Proteobacteria comparing with MCG. Moreover, the diversity of gut microbiota was restored by Shenfu decoction. To connecting host inflammatory response, the intestinal integrity and gut microbiota, previous study had shown that functional lactic acid bacteria induce IL-6 production by dendritic cells contributing to upregulating the sIgA concentration at mucosal sites in humans. Therefore, it is possible that host inflammatory response, the intestinal immunological barrier and gut microbiota are interrelated and interact on each other contributing to the progress of sepsis. However, this study did not further explore how Shenfu decoction regulates sIgA in intestinal mucosal barrier through intestinal flora. This study explored the possible mechanism of Shen Fu Decoction in treating sepsis from reducing inflammation and alleviating gut immunity as well as intestinal flora disturbance. Shenfu decoction decreased the mortality and reduced the peritoneal inflammation of sepsis rats; ameliorated the sepsis-induced serum TNF-α, IL-6 and D-lactic acid levels; improved the morphology of intestinal mucosa; increased the concentration of the sIgA at mucosal; and regulated gut microbiota dysbiosis induced by sepsis. These results support the possible protective role of Shenfu decoction as a supplementary therapeutic target for the sepsis. These findings provided a comprehensive understanding of the protective effects of Shenfu decoction on the host inflammatory response, intestinal immunological barrier and gut microbiota dysbiosis in sepsis rats. However, we only selected a fixed dose of Shenfu decoction while any dose-dependent effects were required for further investigations. Due to distinctive metabolisms of herbal medicines, we did not compare the efficacy of intravenously injected Shenfu injection and orally administered Shenfu decoction. A more comprehensive study design and detailed investigations of molecular mechanisms are indeed essential for the understanding of Shenfu decoction in sepsis. In conclusion, Shenfu decoction may play a protective role in rats with CLP-induced sepsis, by decreasing inflammation, improving intestinal immunological barrier, and regulating gut microbiota. This study may provide an insight into the mechanism underlying its action against sepsis.

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