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ORIGINAL ARTICLE

The Protective Effects and the Involved Mechanisms of Tanshinone IIA on Sepsis-Induced Brain Damage in Mice

Cun-quan Xiong,[1] Hong-cheng Zhou,[1] Jian Wu,[2,3] and Nai-Zhou Guo[2,3]

Abstract—To evaluate the protective effect of tanshinone IIA on sepsis using a mouse model as well as to preliminarily explore the mechanism behind its application. The mouse model of sepsis was established using the cecal ligation and puncture (CLP) method. Eighty mice were randomly divided into four groups: Sham operation group (Sham group), model group (CLP group), tanshinone IIA group (DS group), and dexamethasone group (DEX group). ELISA method was used to detect the levels of TNF-α and IL-6 in the hippocampal tissue of mouse. Western blot method was used to detect the expression levels of PSD-95, SYP, and Iba-1 in the hippocampus tissue. Immunohistochemistry was used to detect the expression level and distribution of astrocytes (GFAP antibody). Morris water maze test was used to determine the ability of learning and memory in mice. Tanshinone IIA could improve the postoperative survival and 7-day survival rate in the septic mice after operation, which shortens the escape latency and increases the number of crossing platform in the septic mice. It also reduces the expression of TNF-α, IL-6, and Iba-1 in the peripheral blood/hippocampus and the number of astrocytes in hippocampal CA3 area after 7 days of sepsis in mice. However, tanshinone IIA increases the expression levels of SYP and PSD-95 in the hippocampus of septic mice on the seventh day after operation. Tanshinone IIA has a protective effect on the nerve of septic mice, and its mechanism may be related to the anti-inflammatory effects of the peripheral and hippocampal parts as well as inhibiting the over-activation of astrocytes and microglia.

KEY WORDS: tanshinone IIA; sepsis; cecal ligation and puncture; protective effect.

INTRODUCTION

Sepsis is a common complication in the intensive care unit. It is dangerous and can rapidly develops into a multiple organ dysfunction syndrome [1, 2]. It severely damages multiple organs such as the brain, heart, and lungs. The clinical treatment is poor and the mortality is extremely high. Sepsis is a systemic inflammatory response syndrome caused by multiple pathogens and involves multiple organs. The brain is the earliest and the most vulnerable tissue of sepsis, and clinically, it is known as sepsis-associated encephalopathy (SAE) [3, 4]. SAE may be related to inflammatory response, dysfunction of neurons, abnormal glial cells, destruction of blood brain barrier, cerebral vascular dysfunction, neurotransmitter disorder, neuron apoptosis, and mitochondrial dysfunction [3–6].

The animal model is an essential experimental platform for the study of sepsis [7]. The commonly used methods for establishing animal models of sepsis currently include the destruction of inherent protective barrier of animal, the leakage of bacteria-containing contents, and the displacement of bacteria. Such as intestinal fistula and

[1] College of pharmacy, Jiangsu Vocational College Medicine, Yancheng, 224002, Jiangsu, China
[2] Department of Laboratory Medicine, The First People's Hospital of Yancheng City, 166 Yu Long Road, Yancheng, 224005, Jiangsu, China
[3] To whom correspondence should be addressed at Department of Laboratory Medicine, The First People's Hospital of Yancheng City, 166 Yu Long Road, Yancheng, 224005, Jiangsu, China. E-mails: piaoxue1982717@sina.com; gnz120@163.com

peritonitis, commonly used methods are cecal ligation and puncture (CLP) and continuous abdominal drainage catheter (colon ascendens stent peritonitis, CASP), intravenous injection of bacteria (including viable and inactivated bacteria) or endotoxin.

Tanshinone IIA is the main component of *Salvia miltiorrhiza* which is one of the traditional Chinese medicines. An earlier study indicated that tanshinone IIA has anti-inflammatory, free radical-scavenging, and enhanced antioxidant activity and, thus, has a protective effect on different types of cardiovascular and cerebrovascular diseases [8]. Tanshinone IIA can protect the myocardium of septic rats by anti-inflammatory, antioxidative, improved apoptosis-related genes and abnormal expression of calcineurin. Studies have also shown that tanshinone IIA can reduce lung injury in septic rats by inhibiting lipid peroxidation and scavenging oxygen free radicals and by its anti-inflammatory effects [5]. However, there are only few studies which focused on the neuroprotective effect of tanshinone IIA on sepsis and its specific mechanisms.

Based on this, in this study, a CLP method was used to establish a sepsis model in mouse and drug intervention. Through the detection in the survival rate of mouse, learning cognitive ability, brain neurons, and glial activation, the inflammatory factors to evaluate the protective effect of tanshinone IIA on the sepsis model using mice as well as its related mechanism were preliminarily explored.

MATERIALS AND METHODS

Experimental Animals

The healthy C57BL6 male mice (8~10 weeks old, 25~30 g, certificate number 2015000500367) used were produced by Shanghai SLEAK Laboratory Animal Co., Ltd. All mice were adapted for 2 weeks before the experiment at the Experimental Animal Center of Jiangsu Medical Vocational College. All animal experiments were approved by the Committee on the Ethics of Animal Experiments of Jiangsu Medical Vocational College and performed strictly with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Establishment of a Mouse Model of Sepsis

The mouse model of sepsis was established using the CLP method. All mice were fasted for 12 h before surgery, but allowing free drinking water, and after intraperitoneally administering 400 mg/kg of chloral hydrate anesthesia, they were fixed and disinfected and a median abdomen incision of about 1~1.5 cm was made layer by layer in order to expose the cecum. To avoid intestinal obstruction, ligation of 1/2 in the cecum with a 3-0 suture ligation was used. Then, 22G needles were used to run through the middle of the ligation section for two times, squeezing out fewer contents, smearing the intestinal wall, and, then, appending the cecum and suturing them one by one. After surgery, all mice received a subcutaneous injection of 1 ml of saline on the back of the neck to assist their recovery.

Experimental Grouping and Administration

Eighty mice were randomly divided into four groups: (1) Sham operation group (Sham group). All the followed operations were the same as that of other groups except that no ligation or puncture was performed. At the same time, after the operation, the mice in this group were given an equal dose of physiological saline within 1 week (the dose was referenced to DS group).

(2) Model group (CLP group): After modeling, the intervention method was the same as that of the Sham group.

(3) Tanshinone IIA group (DS group): After modeling, Tanshinone IIA was given 40 mg/kg at the same time.

(4) Dexamethasone group (DEX group): After modeling, dexamethasone injection of 1.2 mg/kg was given at the same time.

The Survival Time and the 7-Day Survival Rate of Mice in Different Periods After Operation

After surgery at different times, the survival status of all mice was recorded which include activity, change in weight, hair, rectal temperature, appetite, stool frequency and traits, intra-abdominal infection, and inflammation. At the end of surgery, the above groups of mice were recorded as 0 day, and the changes in the number of dead mice were recorded 7 days after the operation, and then, the survival curves were plotted.

Collection of Brain Specimen

Seven days after surgery, the brain was decapitated after perfusion with approximately 40 ml of PBS. Half of the surviving mice in each group were isolated from the hippocampus and stored in a refrigerator at − 86 °C. The other half of the surviving mice in each group were perfused; the brains were fixed with about 20 ml of 8%

paraformaldehyde, and the brains were taken out and soaked in paraformaldehyde solution (8%) and stored in a refrigerator at 4 °C for at least 24 h.

Detection of the Levels of TNF-α, IL-6 in Mouse Hippocampal Tissue Using ELISA Method

The above hippocampus tissue kept at − 86 °C in the refrigerator was removed, homogenized by the addition of PBS (10:1), and centrifuged at a low temperature (3000 rpm, 20 min). Then, the supernatant was collected on ice and stored at − 20 °C in a freezer. ELISA kits (R&D systems company, USA) were used to detect the levels of TNF-α and IL-6 in the hippocampus tissue.

Detection on the Expression Levels of PSD-95, SYP, and Iba-1 in the Hippocampus Tissue Using Western Blot Method

The hippocampus tissue was homogenized and lysed by RIPA lysate (10:1) and centrifuged at low temperature (14,000 rpm, 45 min). The supernatant was drawn and the concentration of protein was determined by BCA method. After adding the loading buffer, it was mixed well and then boiled for 4~5 min. After complete cooling, it was then subjected to centrifugation. The prepared specimens were loaded in the order of the Sham group, CLP group, DEX group and DS group and separated by SDS-PAGE gel electrophoresis and then transferred to a wet membrane. Skimmed milk powder of 5% was blocked for 1 h and incubated with a primary antibody at 4 °C overnight. After washing with TBS for three times, the secondary antibody was incubated at room temperature for 1 to 1.5 h. After washing with TBS for three times, ECL was used for color development and compression. The film was scanned and then analyzed quantitatively by using Image J software.

Detecting the Expression Level and Distribution of Astrocytes (GFAP Antibody) Using Immunohistochemistry

The brain tissue of each group was removed from the fixative and was washed twice with PBS, dehydrated with a gradient of 21 and 30% sucrose, and then embedded in OCT. The temperature of the slicer was set at − 23 °C, and the slice thickness was 30 μm. Frozen sections were immersed in PBS, placed in 80% glycerol solution, and stored at − 20 °C in a freezer.

DAB assay was used to determine the content and distribution of astrocytes (GFAP antibodies, Cell Signaling Technology). From each group of frozen sections, the sections corresponding to the nerve anatomy were selected and blocked by PBS for 1 to 2 h. The primary antibody was incubated at 4 °C overnight. The sections were placed in PBS and dipped at 4 °C, and the secondary antibody was incubated at 4 °C for 1.5 to 2 h. The sections were then placed in PBS and dipped at 4 °C for more than 0.5 h. All sections were simultaneously placed in the DAB reaction solution and 1% H_2O_2 was added to initiate the color reaction. After washing, patching, and dehydrating with different concentrations of alcohol, xylene is degreased and transparent and, then, dropped into a neutral gum seal. Photographed by the microscope, astrocytes were automatically counted by an image processing software, ImageJ.

Determination on the Ability of Learning and Memory in Mice Using Morris Water Maze Test

Another 80 mice were taken and the Morris water maze experiment started on the 7th day after surgery, which includes positioning navigation and space exploration tests. The positioning test was started on the second day after 4 consecutive days of training. Each mouse was recorded from the time it was placed into water to stand on the platform, which was referred as the escape latency. If the platform was not found within the defined time (60 s), it was recorded as 60 s. It was measured four times a day continuously for 5 days, and the average value was considered. After the completion of positioning voyage test, the experiments on space exploration were conducted at an interval of 1 day. The platform was withdrawn and the mouse was placed in water at any location, and the number of times it crosses, the quadrant of the original platform was recorded within 60 s.

Statistical Methods

All the data obtained from the experimental results were analyzed using the statistical software, SPSS 18.0. Data are presented as the mean ± standard deviation when data were normally distributed or as medians and range if the distribution was skewed. The mean comparison between multiple groups was analyzed by analysis of variance (ANOVA). The comparison between groups was performed using LSD test, and the Kaplan-Meier method was used for survival analysis, where the test level was $P < 0.05$.

RESULTS

Tanshinone IIA Improves Postoperative Survival in Septic Mice

Twenty-four hours after surgery, all three groups except the Sham group showed a decreased apathetic activity, an increased rectal temperature, a loss of appetite, dilute vertical hair, wet stool, adhesion at the anal canal, a small amount of intraperitoneal ascites, no odor, cecal congestion, swelling in the ligated section, and no other lesions in other organs in the abdominal cavity (Fig. 1, Table 1).

Forty-eight hours after surgery, the mice in the DEX group recovered their luster and the mice in the CLP and DS groups remained dilute. The rectal temperature in the DS and the DEX groups decreased (Fig. 1). The appetite and hence the activity increased. The CLP group had no obvious changes (rectal temperature continuously increased). Mice in the CLP and DS groups had shown diarrhea. The appearance of stool in DEX group recovered to normal. In the CLP group, there were more bloody/turbidity ascites in the abdominal cavity of mice, with stink, swelling and blood stasis in the cecum and surrounding intestines, and mild congestion in the liver and lungs. In the DS and DEX groups, the blood was aggravated by the cecum and its surrounding tissues.

Seventy-two hours after surgery, the hair of mice in the DS group recovered, and the mice in the CLP group remained dilute. The rectal temperature of mice in the DS and DEX groups returned to normal, and the CLP group remained high (Fig. 1).

Mice in the CLP group had massive abdominal blood/turbid water, odor, pericecal tissue, extensive adhesions between tissue and abdominal wall, swollen bowel, and marked congestion of lung and liver. In the DS and DEX groups, the cecal necrosis of mice was accompanied by a small amount of ascites, which slightly adhered to the surrounding intestine and abdominal wall. The stool condition was similar to that of 48 h.

Seven days after surgery, the body temperature, mental, and activity levels of mice in the other groups returned to normal, except for CLP group which had higher body temperature. Except for the Sham group, the other groups had obvious masses (non-slip) in the abdomen as well as low appetite. The mice in the CLP group had extensive adhesion and congestion in the abdominal viscera, a large amount of yellow green ascites, foul smell, and intestinal erosion. There were adhesions in the abdominal cavity near the cecum and the abdominal wall of mice in the DEX and DS groups. Ascites was for more than 72 h, which was accompanied by blood/turbidity and malodor. In case of mice in the DEX and DS groups, the abdominal cavity near the cecum part of the intestinal and abdominal wall adhesions, where ascites was more than 72 h, was bloody/turbid and smelly.

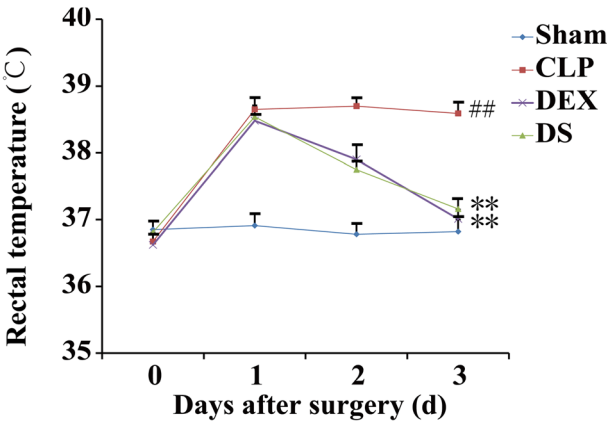

Fig. 1. Comparison of rectal temperature of mice among different groups 1~3 days afte DXM group returned to normal, while that in the CLP group was still significantly higher than that in Sham group. $^{\#\#}P < 0.01$ compared with Sham group; $^{**}P < 0.01$ compared with CLP group. Data are presented as mean ± SD, $n = 10$. CLP cecal ligation and puncture, DEX dexamethasone, DS tanshinone IIA.

Tanshinone IIA Improves 7-Day Survival Rate in the Sepsis Mice After Operation

Seven days after surgery, all mice in the Sham group survived. The survival rate in the CLP group was 51.3%, which was significantly lower compared to the Sham group ($P < 0.01$). The 7-day survival rate in the DEX group was 82.8%, which was higher than the CLP group ($P < 0.05$), and the 7-day survival rate for mice in the DS group was 89.9% which was higher than the CLP group ($P < 0.01$) (Fig. 2).

Tanshinone IIA Shortened the Escape Latency and Increased the Number of Crossing Platform in Septic Mice

During 1~5 days of localization cruising test, the escape latency of mice in the CLP group was longer than that of Sham group ($P < 0.05$, $P < 0.01$), and in the DS group, it was shorter than the CLP group ($P < 0.05$, $P < 0.01$), but there was no significant difference between DEX and CLP groups ($P > 0.05$).

Table 1. Comparison of Survival State of Mice in Different Periods After Operation ($n = 10$)

	Hair				Activity				Appetite			Stool			Intraperitoneal infection and inflammation			
	Sham	CLP	DEX	DS	Sham	CLP	DEX	DS	Sham	CLP	DEX	DS	Sham	CLP	DEX	DS	Sham	CLP	DEX	DS
24 h	–	II	II	II	–	II	II	II	–	II	II	II	–	II	II	II	–	I	I	I
48 h	–	II	I	II	–	II	I	I	–	II	I	I	–	III	–	II	–	III	II	II
72 h	–	III	–	I	–	II	–	–	–	III	–	–	–	III	–	–	–	IV	III	III
7 days	–	IV	–	–	–	III	–	–	–	III	–	–	–	III	–	–	–	V	IV	IV

Hair classification—normal, I: sparse hair on the back; II: sparse hair on the back and head; III: sparse hair on the back, head, and abdomen; IV: sparse hair of the whole body. Activity classification—normal, I: activity reduced, but still active after touching; II: activity decreased and remained inactive after touching; III: difficulty of activity. Appetite classification, according to the daily food intake of mice: 7–8 g/day (normal), I, 5–6 g/day; II, 3–4 g/day; III, 1–2 g/day; IV, 0 g/day. Stool classification—normal, I: increasing frequency; II: humid and adhesion; III: increasing frequency, humid, and adhesion. Classification of infection and inflammation—normal, I: a small amount of ascites or slight congestion of the cecum; II: moderate amount of ascites or moderate congestion of the cecum; III: a large amount of ascites, or severe congestion of the cecum and surrounding intestinal congestion, or mild adhesion of the cecum and surrounding tissues; IV: a large amount of bloody and smelly ascites, or extensive adhesion of the cecum and surrounding tissues; V: a large amount of yellowish green and smelly ascites, or easily broken intestines
CLP cecal ligation and puncture, *DEX* dexamethasone, *DS* tanshinone IIA

In the space exploration experiment, the number of crossing platforms of mice in the CLP group was significantly lower than the Sham group ($P < 0.01$), and the DS group was significantly more than the CLP group ($P < 0.01$), but there was no significant difference between the DEX and CLP groups ($P > 0.05$) (Table 2).

Tanshinone IIA Reduces the Expression of TNF-α and IL-6 in the Peripheral Blood and Hippocampus After Sepsis in Mice

After surgery, the levels of inflammatory cytokines TNF-α and IL-6 in the hippocampus of the CLP, DEX, and DS mice continued to increase (Fig. 3). On the seventh day after surgery, the levels of TNF-α and IL-6 in the hippocampus of the CLP group were higher than in the Sham group ($P < 0.01$). Compared to CLP group, TNF-α and IL-6 were increased in the DEX and DS groups ($P < 0.05$, $P < 0.01$), and in the DS group, it decreased more significantly ($P < 0.01$) (Tables 3 and 4).

Tanshinone IIA Improves the Expression Level of SYP and PSD-95 in the Hippocampus of Septic Mice on the Seventh Day After Operation

Seven days after surgery, the levels of SYP and PSD-95 in the hippocampal tissue of the CLP group were significantly lower compared to Sham group ($P < 0.01$),

Fig. 2. Comparison of survival rate of mice among different groups 7 days after surgery ($n = 20$). Seven days after surgery, the survival rates of Sham group, CLP group, DEX group, and DS group were 100%, 51.3%, 82.8, and 89.9%, respectively. The survival rates of the DS group and DEX group were significantly higher than those of the CLP group. $^{\#\#}P < 0.01$ compared with sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the CLP group. CLP cecal ligation and puncture, DEX dexamethasone, DS tanshinone IIA.

Table 2. Comparison of the Average Escape Latency and the Times of Crossing Platform in each Group of Mice ($n = 10$)

Groups	Day1	Day 2	Day 3	Day 4	Day 5	Times of crossing platform
Sham	45.8 ± 4.2	35.8 ± 3.5	26.5 ± 3.2	21.3 ± 3.1	19.4 ± 3.6	3.7 ± 0.7
CLP	54.7 ± 8.9$^{\#}$	50.9 ± 4.4$^{\#}$	44.2 ± 5.9$^{\#\#}$	36.4 ± 3.5$^{\#}$	33.7 ± 4.4$^{\#}$	1.9 ± 0.6$^{\#\#}$
DEX	50.6 ± 8.1	48.2 ± 2.5	42.2 ± 2.7	33.8 ± 3.0	31.2 ± 2.8	2.4 ± 0.5
DS	47.9 ± 2.2*	40.1 ± 3.5**	29.9 ± 3.3*	23.5 ± 2.9*	20.6 ± 3.1*	3.0 ± 0.5**
F	15.946	36.390	75.374	57.190	42.883	19.053
P	0.000	0.000	0.000	0.000	0.000	0.000

$^{\#\#}P < 0.01$, $^{\#}P < 0.05$ compared with the Sham group; $^{**}P < 0.01$, $^{*}P < 0.05$ compared with the CLP group. Data are presented as mean ± SD, $n = 10$
CLP cecal ligation and puncture, *DEX* dexamethasone, *DS* tanshinone IIA

and the levels of SYP and PSD-95 in the DS group were higher compared to the CLP group ($P < 0.01$, $P < 0.05$). There was no significant difference between the DEX and CLP groups ($P > 0.05$) (Fig. 4).

Tanshinone IIA Reduces the Number of Astrocytes in the Hippocampal CA3 Area After 7 Days of Operation in Septic Mice

Seven days after surgery, the number of astrocytes in the hippocampal CA3 region in each group of mice was significantly increased as compared to the Sham group ($P < 0.01$). In both the DEX and DS groups, they were less than the CLP group ($P < 0.01$) (Fig. 5).

Tanshinone IIA Reduces the Expression of Iba-1 in the Hippocampus of Septic Mice After 7 Days of Operation

Seven days after surgery, the expression level of Iba-1 in the hippocampus of CLP group mice was significantly higher compared to the Sham group ($P < 0.01$), but significantly decreased in the DEX and DS groups ($P < 0.05$, $P < 0.01$) (Fig. 6).

DISCUSSION

The CLP model has the characteristics of clinical perforation of acute appendicitis and is currently the most widely used model and is well recognized as the gold standard of the sepsis model [9]. The CLP model has the following advantages: (1) The occurrence of sepsis is induced by the autoinfection of experimental animals, which is consistent with the natural course of human infection and can produce pathophysiological changes similar to human sepsis, such as changes in the pro-inflammatory factors of IL-6 and TNF-α. Organ damage, different stages of hemodynamics and metabolic changes (early high-ranking low resistance, higher metabolism, late low row low resistance), cell selective apoptosis, and biochemical changes. Studies have shown that after 6 h of modeling in the septic mice, the level of IL-6 could change, and the mortality rate of mice increases immediately. This phenomenon is very similar to the biochemical characteristics of human sepsis. (2) Animals have a long survival time and can simulate the treatment of clinical sepsis at the same time as that of modeling, such as the use of

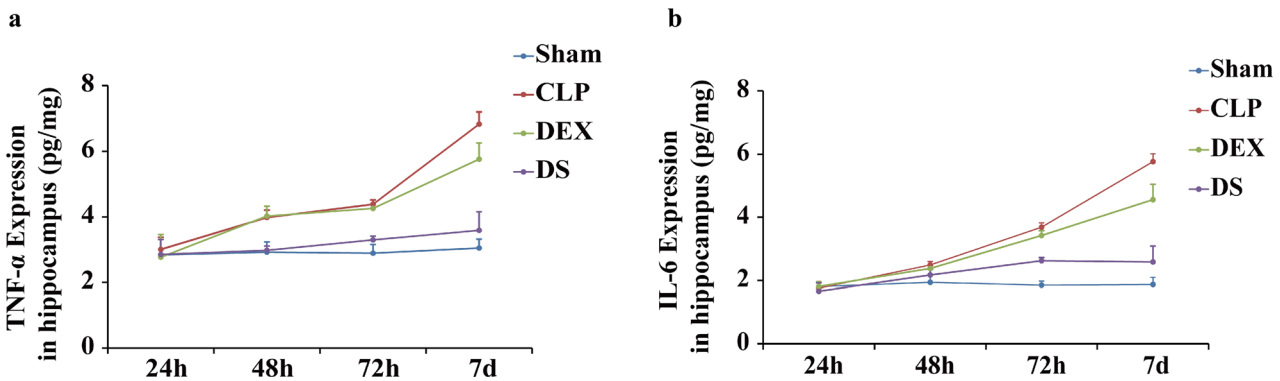

Fig. 3. Changes of inflammatory cytokine levels in the hippocampus of the mice brain in each group with different times after surgery. The expression of inflammatory cytokine in the hippocampus was detected by an ELISA kit at different time points after operation. It was found that the levels of TNF-α (**a**) and IL-6 (**b**) in the other three groups were continuously elevated except the Sham group. Data are presented as mean ± SD, $n = 3$. CLP cecal ligation and puncture, DEX dexamethasone, DS tanshinone IIA.

Table 3. The Expression Levels of TNF-α in the Hippocampus of Mice in each Group with Different Times After Surgery (pg/mg, $n = 3$)

Group	24 h	48 h	72 h	7 days
Sham	2.84 ± 0.46	2.92 ± 0.31	2.89 ± 0.27	3.05 ± 0.05
CLP	2.00 ± 0.37	3.98 ± 0.23$^{\#\#}$	4.38 ± 0.13$^{\#\#}$	6.83 ± 0.37$^{\#\#}$
DEX	2.77 ± 0.69	4.02 ± 0.30$^{\triangle\triangle}$	4.26 ± 0.12*$^{\triangle\triangle}$	5. 76 ± 0.50*$^{\triangle\triangle}$
DS	2.85 ± 0.45	2.98 ± 0.13**	3.29 ± 0.12**	3.59 ± 0.57**
F	0.114	17.182	117.237	63.552
P	0.949	0.001	0.000	0.000

$^{\#\#}$ $P < 0.01$ compared with the Sham group; $^{**}P < 0.01$, $^{*}P < 0.05$ compared with CLP group; $^{\triangle\triangle}$ $P < 0.01$ compared with the DS group. Data are presented as mean ± SD, $n = 3$

CLP cecal ligation and puncture, *DEX* dexamethasone, *DS* tanshinone IIA

antibacterial drugs and liquid resuscitation, and are suitable for the research and development of therapeutic drugs causing severe sepsis; (3) the modeling cost is low, process of operation is simple, repeatability is high, and controllability is good. By controlling the length of cecal ligation and the size and number of punctures, different degrees of the risk of sepsis model and multiple organ dysfunction could be induced. Therefore, this study selected CLP method to establish a mouse model of sepsis and to induce damage of brain tissue. The results showed that the mortality of mice in the CLP group was as high as 48.3% at 7 days after surgery, and the activity was significantly reduced, along with apathy, decreased learning and memory, an abnormal increase in the inflammatory transmitters in the peripheral blood and brain, and decreased neurons. The clinical manifestations of central nervous system in patients with severe sepsis have provided a good basis for the study of the neuroprotective effect of tanshinone IIA on the mouse model of sepsis.

Sepsis is a type of disease with a high mortality rate [10]. In this study, CLP model which is similar to pathophysiology of human sepsis was selected (selecting the middle cecum ligation to produce a moderate sepsis model) to study the effects of tanshinone IIA on sepsis. Glucocorticoids are a class of clinically used drugs that have powerful anti-inflammatory effects and can be combined with effective antibiotics for septic shock [11]. However, the use of glucocorticoids for the treatment of sepsis is still controversial. Most clinical trials have shown that small doses of hydrocortisone might increase the survival rate of patients with sepsis. Few researchers have found that glucocorticoids have no significant effect on the mortality of patients with sepsis and may even increase the possibility [12]. This uncertainty in the treatment of sepsis by glucocorticoids has been recognized in the treatment guidelines of sepsis around the world. In China, glucocorticoids with low dosage are also included in the treatment guidelines for sepsis and recommended for use with adrenocortical function incomplete sepsis patients [13]. In this study, dexamethasone was selected as a positive control to study the protective effect of tanshinone IIA using mice as a sepsis model. Survival time and its rate are the most intuitive indicators for assessing the changes in the conditions of CLP mice after different treatments. The experimental results show that tanshinone IIA and dexamethasone can improve the survival rate of septic mice, where in the DS group, it increased more significantly. In addition, tanshinone IIA and dexamethasone can improve the

Table. 4. The Expression Levels of IL-6 in the Hippocampus of Mice in each Group with Different Times After Surgery (pg/mg, $n = 3$)

Group	24 h	48 h	72 h	7 days
Sham	1.80 ± 0.16	1.94 ± 0.25	1.85 ± 0.13	1.87 ± 0.23
CLP	1.76 ± 0.21	2.49 ± 0.11$^{\#}$	3.68 ± 0.14$^{\#\#}$	5.76 ± 0.25$^{\#\#}$
DEX	1.81 ± 0.14	2.38 ± 0.09$^{\triangle}$	3.42 ± 0.15*$^{\triangle\triangle}$	4.56 ± 0.49*$^{\triangle\triangle}$
DS	1.66 ± 0.26	2.17 ± 0.18*	2.62 ± 0.11**	2.58 ± 0.50**
F	0.363	6.052	117.237	63.552
P	0.782	0.019	0.000	0.000

$^{\#\#}$ $P < 0.01$, $^{\#}$ $P < 0.05$ compared with the Sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the CLP group. $^{\triangle\triangle}$ $P < 0.01$, $^{\triangle}$ $P < 0.05$ compared with the DS group. Data are presented as mean ± SD, $n = 3$

CLP cecal ligation and puncture, *DEX* dexamethasone, *DS* tanshinone IIA

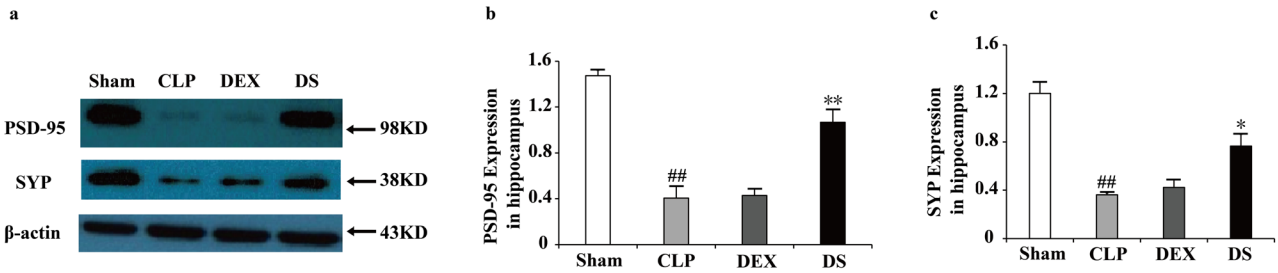

Fig. 4. The expression levels of PSD-95 and SYP in the hippocampus of mice in each group 1 week after surgery. After protein quantitation, Western blot was performed to evaluate PSD-95 and SYP expression in the hippocampus. **a** Expression of PSD-95 and SYP protein bands in hippocampus. **b** Expression ratio of PSD-95 and β-actin in the hippocampus. **c** Expression ratio of SYP and β-actin in the hippocampus. The expression of PSD-95 and SYP in the hippocampus of the CLP group was significantly lower than that of the Sham group, and the expression of these two proteins in the DS group was significantly higher than that in the CLP group. $^{\#\#}P < 0.01$ compared with Sham group; $^{*}P < 0.05$, $^{**}P < 0.01$compared with CLP group; data are presented as mean ± SD, $n = 3$. CLP cecal ligation and puncture, DEX dexamethasone, DS tanshinone IIA.

postoperative general performance of septic mice such as reducing the rectal temperature, increasing the appetite, and inhibiting the intra-abdominal inflammatory response. In summary, tanshinone IIA and DEX have protective effects on septic mice, and notably, the protective effect of DS is even more pronounced.

Studies have shown that CLP-induced sepsis in rat brain neurons were significantly reduced in certain regions and decreased more obviously the in brain areas including the hippocampus (CA1, CA3, and dentate gyrus), hypothalamus median preoptic nucleus, and subventricular zone which are associated with apoptosis of neurons caused by mitochondrial dysfunction [10]. Synaptophysin (SYP) in the presynaptic membrane, and postsynaptic density (PSD-95) in the postsynaptic membrane are synaptic-specific molecular markers which are widely distributed in the brain [14, 15]. The hippocampus and the cortex maintain the normal function and plasticity of synapses. Thomas et al. [16] believed that the release of neurotransmitters is related to the phosphorylation of SYP when neurons are excited. SYP plays a significant role in the repair of neuronal damage, and the number of changes can reflect the degree of synapse formation and remodeling during neuronal damage [17].

Studies have found that an abnormal expression of SYP is associated with neuropsychiatric disorders such as depression, brain injury, and ischemic encephalopathy [18]. PSD-95 is an excitatory protein with a molecular weight of 95 kDa which is located in the postsynaptic membrane of glutamatergic neurons and is composed of

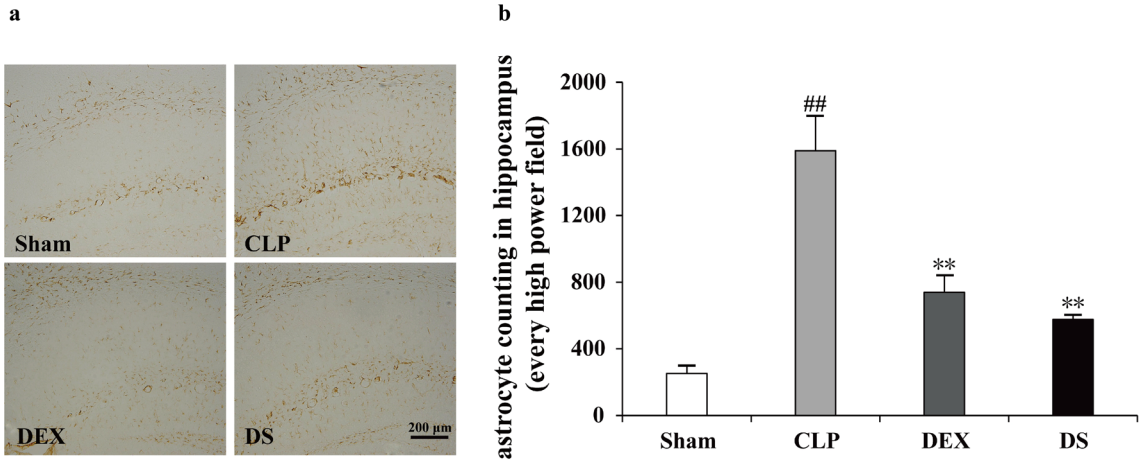

Fig. 5. Astrocyte counting in the hippocampus of mice in each group 7 days after surgery ($n = 3$). Immunohistochemical method was used to detect the expression of microglia in the hippocampus of mice in each group. **a** After DAB staining, the distribution of microglia in the hippocampus of each group of mice was observed. **b** Microglia counts in the hippocampus of each group of mice. The expression of astrocytes in the hippocampus of the CLP group was significantly higher than that of the Sham group, and the expression of astrocytes in the DS group and DEX group was significantly lower than that of the CLP group. $^{\#\#}P < 0.01$ CLP group *VS* Sham group; $^{**}P < 0.01$ compared with the CLP group. CLP cecal ligation and puncture, DEX dexamethasone, DS tanshinone IIA. Bar, 200 μm.

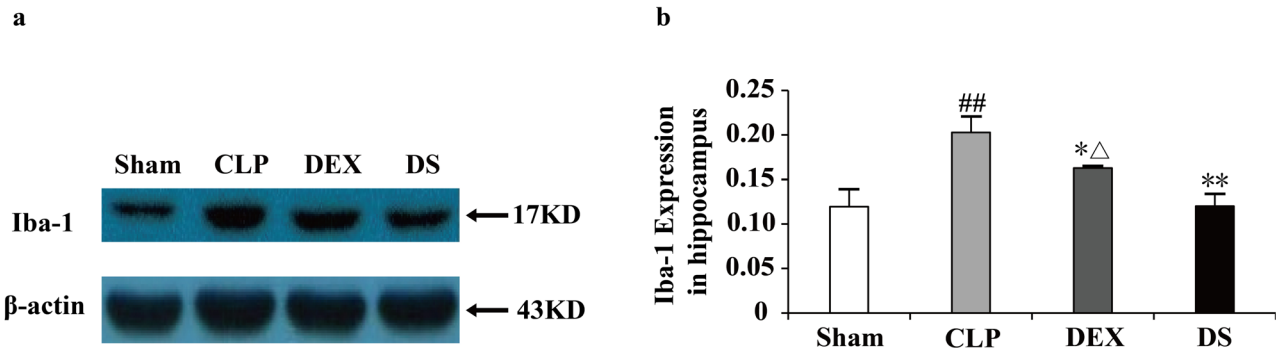

Fig. 6. The expression levels of Iba-1 in the hippocampus microglia of mice in each group 7 days after surgery. After protein quantitation, Western blot was also performed to evaluate Iba-1 expression in the hippocampus. **a** Expression of Iba-1 protein bands in the hippocampus. **b** Expression ratio of Iba-1 and β-actin in the hippocampus. The expression of Iba-1 in the CLP group was significantly higher than that in the Sham group. The expression of Iba-1 in the DS group and DEX group was significantly lower than that in the CLP group and decreased significantly in the DS group. $^{\#\#}P < 0.01$ CLP group *VS* Sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the CLP group; $^{\triangle}P < 0.05$ compared with DS group. Data are presented as mean ± SD, $n = 3$. CLP cecal ligation and puncture, DEX dexamethasone, DS tanshinone IIA.

receptors, signal regulators, and scaffold proteins [19]. It is involved in many of the physiological processes of the body. Studies have shown that an abnormal expression of PSD-95 occurs in rats with blunt brain injury, which is related to the conduction of damaged signals [20]. In the mouse model of senile dementia, the levels of PSD-95 in the brain of mice were greatly reduced with learning and memory, with a decline in the learning and memory ability. The results of this study indicate that the number of neurons in the hippocampal CA3 region of cerebral tissue in mice with sepsis in the CLP group is reduced. This demonstrates that the established sepsis model does have pathological lesions in the brain, and the hippocampal tissue damage is more obvious—the CLP group sepsis. The expression levels of SYP and PSD-95 in mice were significantly decreased, showing that the function of synapses was impaired and the number of neurons in the hippocampus of the sepsis group was increased as compared to CLP group, as well as the expression levels of SYP and PSD-95 were increased. These results indicate that tanshinone IIA can protect the brain tissue of septic mice which is related to its promotion of synaptic remodeling.

In patients with sepsis complicated by brain lesions, cognitive and disorientation, unconsciousness, and other neurological manifestations may occur. Among them, cognitive impairment is the most common manifestation which can persist for a long time after the patient is discharged from the hospital and greatly reduces the quality of life and working condition of the patient [21]. Gauthier et al. [22] divided cognitive impairment into amnestic and non-forgetting cognitive disorders. The cognitive impairment in patients with sepsis is mostly the former. Studies have shown that water maze training could make the synapses in rats plastically changing in the morphology and manifested as an increase in the number of synapses, vesicles, and volume, thereby forming memory (including spatial orientation) [23]. Synaptic plasticity is closely related to SYP and PSD-95. In this experiment, Morris water maze test was used to examine the learning and memory function of each group of mice after successful modeling. The experimental results showed that the escape latency of mice in the CLP group was prolonged, and the number of crossing platforms in the space exploration experiment was significantly reduced, indicating that learning and spatial memory ability of the sepsis mice were decreased, and cognitive impairment existed, while the mice in the DS group escape latency. The shortening and an increase in the number of crossing platforms indicate that tanshinone IIA can improve cognitive function in the septic mice.

It is widely believed that an excessive inflammatory reaction is the main cause of sepsis, indicating that a large number of inflammatory factors are released into the bloodstream and reach multiple organs with blood circulation, leading to multiple organ damage [24]. As the initiator of inflammatory response, TNF-α first appeared in sepsis, and thus, it is often used as an important biomarker for an early diagnosis of the disease [25]. When sepsis occurs, a large amount of TNF-α is released into the blood and activates mononuclear macrophages, causing it to further release a large number of pro-inflammatory factors such as IL-6 and IL-1. When sepsis occurs, TNF-α can enter the central nervous system in large quantities on the basis of the destruction of

blood-brain barrier, which promotes the infiltration of inflammatory cells, apoptosis of neurons and edema of brain tissue, and, ultimately, aggravates brain damage [26]. IL-6 is another important inflammatory factor that can be produced by a variety of cells in the brain [27]. Its main role is to promote the synthesis and secretion of many proteins in the acute phase of inflammation and to act synergistically with TNF-α in the inflammatory response of the central nervous system. It assists clinicians in the early diagnosis of sepsis.

An increased expression of IL-6 in the brain is associated with impaired cognitive function, loss of neurons, and increased mortality in patients with sepsis. Several studies have shown that an excessive activation of microglia is closely related to the diseases of the central nervous system such as Alzheimer's disease [28]. When microglia is activated, its marker Iba-1 expression increases accordingly.

Our study also showed that the inflammatory factors in the hippocampus of the peripheral blood and brain tissues of mice in the CLP group were significantly higher as compared to the Sham group, and astrocytes and microglia were also activated in large amounts. The inflammatory factors in the peripheral blood and hippocampus of DS and DEX groups were lower than in the CLP group. It is to be noted that in the peripheral blood, the DEX group decreased more significantly. While in the hippocampus, the DS group decreased better. This shows that the peripheral anti-inflammatory effect of dexamethasone is stronger compared to tanshinone IIA, while the central anti-inflammatory effect of tanshinone IIA is stronger. The activation of astrocytes and microglia in the DS and DEX groups was reduced, and the DS group was more effective, which shows that both tanshinone IIA and dexamethasone can inhibit the activation of astrocytes and microglia and tanshinone IIA has a stronger effect.

In summary, the neuroprotective effect of tanshinone IIA on a mouse model of sepsis may be related to the anti-inflammatory effects of peripheral and hippocampal sites and the inhibition of over-activation of astrocytes and microglia.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

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