Sodium tanshinone IIA sulfonate attenuates cardiac dysfunction and improves survival of rats with cecal ligation and puncture-induced sepsis

MENG Zheng-Jie¹,², WANG Chao³, MENG Ling-Tong², BAO Bei-Hua⁴,

WU Jin-Hui²*, HU Yi-Qiao²*

¹ College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 211816, China;
² State Key Laboratory of Pharmaceutical Biotechnology, Medical School, Nanjing University, Nanjing 210093, China;
³ School of Pharmaceutical Sciences, Nanjing Tech University, Nanjing 211816, China;
⁴ Jiangsu Key Laboratory for High Technology of Traditional Chinese Medicine Formulae Research, College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China

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[ABSTRACT] Cardiac dysfunction, a common consequence of sepsis, is the major contribution to morbidity and mortality in patients. Sodium tanshinone IIA sulfonate (STS) is a water-soluble derivative of Tanshinone IIA (TA), a main active component of Salvia miltiorrhiza Bunge, which has been widely used in China for the treatment of cardiovascular and cerebral system diseases. In the present study, the effect of STS on sepsis-induced cardiac dysfunction was investigated and its effect on survival rate of rats with sepsis was also evaluated. STS treatment could significantly decrease the serum levels of C-reactive protein (CRP), procalcitonin (PCT), cardiac troponin I (cTn-I), cardiac troponin T (cTn-T), and brain natriuretic peptide (BNP) in cecal ligation and puncture (CLP)-induced septic rats and improve left ventricular function, particularly at 48 and 72 h after CLP. As the pathogenesis of septic myocardial dysfunction is attributable to dysregulated systemic inflammatory responses, several key cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10) and high mobility group protein B1 (HMGB1), were detected to reveal the possible mechanism of attenuation of septic myocardial dysfunction after being treated by STS. Our study showed that STS, especially at a high dose (15 mg·kg⁻¹), could efficiently suppress inflammatory responses in myocardium and reduce myocardial necrosis through markedly reducing production of myocardial TNF-α, IL-6 and HMGB1. STS significantly improved the 18-day survival rate of rats with sepsis from 0% to 30% (P < 0.05). Therefore, STS could suppress inflammatory responses and improve left ventricular function in rats with sepsis, suggesting that it may be developed for the treatment of sepsis.

[KEY WORDS] Sodium tanshinone II A sulfonate; Sepsis; Cardiac dysfunction; Cytokine; Cecal ligation and puncture model


Introduction

Sepsis is a pathological state that results from a harmful or damaging host response to infection. Overproduction of host inflammatory cytokines, such as TNF-α and IL-1β, could be induced by invasion of microorganisms into the blood stream or absorption of toxins (such as LPS) in a local site, which up-regulate the expression of other inflammatory cytokines in turn [¹]. Sepsis is characterized by hypotension, hypoperfusion and organ dysfunction, while the abnormal excessive accumulation of multiple inflammatory cytokines contribute to multiple organ failure, hypotension, hypoperfusion and mortality. It is shown that sepsis is the most common cause of death among critically ill patients in non-coronary intensive care units (ICU) [²], and the annual cost of hospital treatment of sepsis is very high. Therefore, sepsis is an important public health problem.

Salvia miltiorrhiza Bunge is traditionally used to treat cardiovascular and cerebral system diseases in China [³-⁵]. Four decades of research has revealed that Tanshinone IIA (TA)
is not only the important quality control compound [6-10], but also a major active compound for *Salvia miltiorrhiza Bunge* and its formulations [11-16]. However, clinical use of TA is limited by its hydrophobicity. Thus, Sodium tanshinone IIA sulfonate (STS), a water-soluble derivative of TA, is developed. It is found that STS possesses similar pharmacological activities to TA and can also be used to treat cardiovascular and cerebral system diseases, such as cardiomyocyte hypertrophy [17], myocardial infarction, myocardial ischemia-reperfusion injury [18], myocardial fibrosis [19], and stroke [20]. Additionally, STS is further found to have multiple pharmacological effects, including anti-dyslipidemia, inhibition of fatty acid beta-oxidation [18], modulation of ROS, anti-fibrotic effect [19], enhancement of mesenteric perfusion [21], and reduction of inflammatory cytokines [22]. These performances reflect the medical application of STS being no longer limited in angio-cardiopathy. Pulmonary edema, pulmonary arterial hypertension [23], hepatitis [24] and diabetic neuropathy [25] have become the new indications of STS.

The abnormal accumulation of various inflammatory cytokines plays a key role in the pathophysiology of sepsis and cardiac dysfunction is the most important cause for mortality in sepsis. Previous studies have shown that STS can reduce the secretion of some inflammatory cytokines and has an excellent protective effect on cardiac myocytes [18]. It seems that STS could be used for the treatment of septic cardiac dysfunction. However, to the best of our knowledge, there are few studies available on the efficacy of STS in sepsis rats, and the mechanisms of the protective effects of STS in septic cardiac dysfunction have not yet revealed. In the present study, the therapeutic capacity of STS on sepsis-induced cardiac dysfunction and sepsis related inflammatory cytokines were systematically evaluated in animal model of cecal ligation and puncture (CLP)-induced sepsis. Furthermore, the effect of STS on the survival rate of the rats with sepsis was also evaluated.

**Materials and Methods**

**Chemicals and reagents**

STS was prepared by Shanghai No.1 Biochemical & Pharmaceutical Co. Ltd., Shanghai, China (Purity 99.1%, Product number: DS-1210001) according to chemical reactions shown in Fig. 1A. Briefly, 2.4 mL of glacial acetic acid and 4 mL of acetic anhydride were added into 1 g TA (Xi’an Honson Biotechnology Co., Ltd., Xi’an, China; Batch number: 100401, Purity 95.9%, Fig. 1B). The mixture was kept at 10 °C with agitation, and 2 mL of the mixed liquor (\(V_{\text{glacial acetic acid}} : V_{\text{acetic anhydride}} = 1 : 1\)) was added into the mixture dropwise. Then the reaction mixture was kept at room temperature for 1 h with agitation. After that, the reaction mixture was poured into equal-volume distilled water very slowly, to which 20 mL of saturated sodium chloride solution was added immediately. The mixture was centrifugated; the semi-finished product was obtained, washed twice with saturated sodium chloride solution, and dried on a water bath. The dried semi-finished product was refluxed with chloroform for 8 h to remove un-sulfonated liposoluble components, and sodium chloride was removed by refluxed with dehydrated alcohol. Finally, the refined STS was prepared by recrystallization using dehydrated methanol, and its purity was analyzed by a Dionex HPLC (Ultimate 3000, Thermo Fisher Scientific, Shanghai, China). The data are shown in Fig. 1C. To prepare the regent used in the present study, 5 mg STS was dissolved into 1 mL of saline as a stock solution. The stock solution was sterilized by passing through a membrane filter with pore size of 220 nm.

ELISA kits for the detection of rat brain natriuretic peptide (BNP), C-reactive protein (CRP), procalcitonin (PCT), cardiac troponin-I (cTn-I), cardiac troponin-T (cTn-T), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), and high-mobility group protein B1 (HMGB1) were purchased from Bio-Swamp Life Science (Shanghai, China).

**HPLC Analysis**

All samples were analyzed by a Dionex HPLC (Ultimate 3000, Thermo Fisher Scientific, Shanghai, China) equipped with a quaternary pump, a vacuum degasser, an UV detector, a column heater-cooler, and Chrome Leon software system. For TA analysis, an ODS2 C18 column (250 mm × 4.6 mm id, 5.0 µm particle size, Hypersil, Thermo Fisher Scientific, Shanghai, China) was used at room temperature, and mobile phase consisted of water, 0.1% phosphoric acid solution and alcohol (\(V/V = 80 : 10 : 10\)) at a flow rate of 1.0 mL·min⁻¹. The detection wavelength was set at 270 nm. For STS analysis, a Hyper OD2S C18 column (250 mm × 4.6 mm id, 5.0 µm particle size) was used at room temperature, and mobile phase consisted of water, 0.1% phosphoric acid solution and alcohol (\(V/V/V = 25 : 75 : 5\)) at a flow rate of 1.0 mL·min⁻¹. The detection wavelength was set at 220 nm. Standard TA and STS were purchased from National Institutes for Food and Drug Control (Beijing, China).

**Cecal ligation and puncture model of sepsis**

All the animals were housed and handled in accordance to a protocol approved by Laboratory Animal Care and Use Committee of Jiangsu Province [SYXX (SU) 2014-0001]. CLP was performed on Sprague-Dawley (SD) specific pathogen-free (SPF) rats (half male and half female) aged 8 weeks (weighing 220–280 g) [26]. All of SPF rats were purchased from Qinglongshan Animal Breeding Center, Nanjing, China, and were reared under SPF conditions at a rodent production facility in Jiangsu Provincial Institute of Materia Medica, (Nanjing, China). The rats were acclimated for 1 week with free access to food and tap water prior to CLP. Briefly, the rats were anesthetized with isoflurane, and the cecum was isolated via laparotomy. Approximately 30% of the cecum from the cecal tip was ligated using a 4-0 silk suture. Cecum was through- and-through punctured with a 21-gauge needle from mesenteric toward antimesenteric direction after ligation and gently squeezed to express approximately 1 mm column of feces. Wound closure was performed by applying simple...
running sutures to the abdominal musculature and skin. In sham-operated rats (Sham group), the cecum was isolated, but neither ligation nor punctured was performed. After surgery, all the rats were immediately received prewarmed normal saline subcutaneously (37 °C, 5 mL/100 g). At the end of the surgical procedure, the rats were returned to cages in a temperature-controlled room (22 °C) immediately, with free access to tap water and food.

Fig. 1  A, The synthetic route of sodium tanshinone IIA sulfonate. B, HPLC analysis for tanshinone IIA. C, HPLC analysis for sodium tanshinone II A sulfonate

Treatment

120 SD rats (SPF grade) were randomly divided into the following four groups and underwent surgery and drug administration accordingly \( (n = 30): \) Sham group (equal volume of normal saline), CLP group (equal volume of normal saline), STS low dose group [CLP + STS low dose, 5 mg·kg\(^{-1}\) (1 mL·kg\(^{-1}\)), equal to the lowest effective dosage in humans], STS high dose group [CLP + STS high dose, 15 mg·kg\(^{-1}\) (3 mL·kg\(^{-1}\)), equal to three times as much as the lowest effective dosage in humans]. All the animals received the first drug administration right after the surgery via caudal vein injection, repeated every 24 h for 3 days. Ten rats from each group were randomly selected at 24, 48, and 72 h after drug administration for determination of cardiac function and measurement of levels of cytokines in heart tissue and serum. Heart tissues were collected for histologic analysis of myocardial damage. Details of heart tissue sample solutions and serum sample preparation and histologic analysis of heart tissues were listed in the following sections.

Determination of cardiac function

The right carotid artery was exposed, and the distal end of the artery was ligatured. A small incision was then made in the artery and the polyethylene (PE)-50 catheter filled with heparin-saline was inserted into the artery, and the catheter was attached to a pressure transducer. After recording the mean arterial pressure (MAP) of the rat for 10 min, the PE-50
catheter was then inserted into the left ventricle of rat to collect hemodynamic parameters, including $\frac{\text{dp}}{\text{dt}}, \frac{-\text{dp}}{\text{dt}}, \text{LVSP}$ and $\text{LVEDP}$. The heart rates of rats were recorded throughout the experiment.

**Measurement of serum biomarkers**

The rats were anesthetized by i.p. injection of $2\% (W/W)$ pentobarbital sodium [40 mg kg$^{-1}$ (2 mL kg$^{-1}$)] and then were sacrificed by bleeding after cardiac function determination experiments, and blood samples were collected. Serum levels of CRP, PCT, BNP, cTn-I, and cTn-T were measured with ELISA assay kits.

**Measurement of myocardial inflammatory cytokines**

100 mg myocardium collected from each of the rats after cardiac function determination experiments was homogenized in 900 µL of precooled normal saline via a homogenizer at 0 °C. Myocardial homogenates were then centrifugated at 6000 r min$^{-1}$ for 10 min at 4 °C. Supernatants were collected for the measurement of cytokines, including IL-1β, IL-6, IL-10, TNF-α, and HMGB1.

**Histologic analysis of myocardial damage**

At the end of cardiac function determination, the rats were killed with an overdose of diethyl ether, and part of heart of each rat was removed for histological study. The hearts were immediately washed in precooled PBS (1 ×, 4 °C) to remove blood clot, fixed in 4% paraformaldehyde at least for 24 h, and then embedded in paraffin. The heart tissues were sectioned at 4-µm thick and mounted on glass slides. The slides were stained with hematoxylin-eosin (H&E). For each rat, at least 10 high power (400 ×) fields were examined. All the sections were examined in a blinded manner. A semiquantitative grading system described by Nyska [27] was employed to evaluate the extent of cardiac inflammation: minimal (grade 1) changes involved 1%–10% of the section; mild (grade 2) involved 11%–40%; moderate (grade 3) involved 41%–80%; and severe (grade 4) involved 81%–100%.

**Survival analysis**

Additional 40 SD rats (SPF grade) were chosen for survival experiment. Grouping ($n = 10$ in each group) and drug administration procedures were the same as above. The treatments lasted for 18 days with no antibiotic treatment provided and survival was monitored every day throughout the experiment.

**Statistical analysis**

All the data were presented as means ± SD (standard deviation). Differences in cardiac function, serum and myocardial inflammatory cytokines among the groups were determined with one-way analysis of variance (ANOVA). Kaplan-Meier survival curves were compared using a log-rank test. A $P$ value < 0.05 was considered statistically significant.

**Results**

**Effects of STS on cardiac function**

To determine the therapeutic effects of STS on myocardial dysfunction in the CLP rats, cardiac hemodynamics were determined, and the results are shown in Fig. 2.

![Fig. 2  Dose-dependent effects of sodium tanshinone II A sulphonate (STS) on the cardiac function during sepsis. STS at doses of 5 and 15 mg·kg$^{-1}$ or equal volume of normal saline were i.v. administered immediately post-CLP or sham surgery. Drug/saline administration was repeated every 24 h for 3 days. The rats were randomly selected for cardiac hemodynamics assay at 24, 48 and 72 h post-CLP or sham surgery. Values are means ± SD, $n = 10$. †$P < 0.05$, ††$P < 0.01$ vs sham group; *$P < 0.05$, **$P < 0.01$ vs CLP group](image-url)
decline of the left ventricular pressure (\(-\text{dp/dt}_{\text{max}}\)), ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and heart rate (HR), remained stable throughout the entire postoperative observation, which indicated that sham operation had no significant effect on cardiac function. Compared with the sham group, the CLP surgery showed damages to the cardiac functions; the longer the postoperative time, the worse the outcome, such as continuing decrease in MAP \((P < 0.01 \text{ at } 24, 48, \text{ and } 72 \text{ h})\) and HR \((P < 0.05 \text{ at } 24 \text{ and } 48 \text{ h}, P < 0.01 \text{ at } 72 \text{ h})\). The hemodynamic parameters in the CLP group also indicated left ventricular dysfunction, such as decreased LVSP \((P < 0.05 \text{ at } 24 \text{ h}, P < 0.01 \text{ at } 48 \text{ and } 72 \text{ h})\), reduced \(\text{dp/dt}_{\text{max}}\) \((P < 0.05 \text{ at } 48 \text{ h}, P < 0.01 \text{ at } 72 \text{ h})\) and \(-\text{dp/dt}_{\text{max}}\) \((P < 0.01 \text{ at } 48 \text{ and } 72 \text{ h})\), and increased LVEDP \((P < 0.01 \text{ at } 24 \text{ h}, P < 0.05 \text{ at } 48 \text{ h}, \text{ and } 72 \text{ h})\). The low dose of STS partially improved cardiac function, showing increased MAP level at 48 h \((P < 0.05)\), increased \(\text{dp/dt}_{\text{max}}\) at 72 h \((P < 0.05)\), increased \(-\text{dp/dt}_{\text{max}}\) at 24 h \((P < 0.05)\) and 72 h \((P < 0.01)\), and decreased LVEDP at 24 h \((P < 0.05)\). Compared with the low dose STS, high dose of STS had much better effects on heart dysfunction, showing significantly increased MAP level \((P < 0.01 \text{ at } 24 \text{ h}, P < 0.01 \text{ at } 48 \text{ h} \text{ and } P < 0.05 \text{ at } 72 \text{ h})\) and significantly decreased LVEDP throughout the whole postoperative period \((P < 0.05 \text{ at } 24, 48, \text{ and } 72 \text{ h})\), and increased \(\text{dp/dt}_{\text{max}}\) \((P < 0.05 \text{ at } 72 \text{ h})\) and \(-\text{dp/dt}_{\text{max}}\) \((P < 0.05 \text{ at } 48 \text{ h} \text{ and } P < 0.01 \text{ at } 72 \text{ h})\). Besides these results, the LVSP value was markedly increased at the 72 h for the high dose STS group \((P < 0.05)\). Thus, it could be concluded that STS had the ability to enhance the left ventricular systolic and diastolic functions.

**Effects of STS on serum biomarkers**

The results of our study indicated that the serum concentrations of CRP and PCT in the CLP rats increased significantly (Figs. 3A and 3B, \(P < 0.05 \text{ at } 24 \text{ and } 48 \text{ h}, P < 0.01 \text{ at } 72 \text{ h})\), while the two biomarkers remained stable in the sham group during the entire postoperative period. Such significant alterations of serum CRP and CLP levels suggested that severe septic status was achieved via CLP operation in our experiment. As shown in Figs. 3A and 3B, STS treatment reduced the the CRP and PCT levels at 48 h after surgery. The low dose STS significantly reduced the plasma concentration of CRP at 48 and 72 h \((P < 0.05)\), and the PCT level at 72 h \((P < 0.01)\). The similarly therapeutic effects were observed in high dose group, with significant reduction of the PCT level at 48 h \((P < 0.05)\) and much lower level of CRP and PCT at each time point compared with that of the low dose group. These results suggested that STS treatment could attenuate the severity of sepsis.

Myocardial dysfunction often accompanies severe sepsis. In order to evaluate this dysfunction in the CLP rats and monitor the therapeutic effects of STS on myocardial dysfunction, cTn and BNP levels were detected in different groups. Although cardiac troponin is composed of three subunits C, I and T, only cTn-I and cTn-T are ideally suited for the detection of myocardial damage since these two cytokines are expressed as cardio-specific isoforms \([28]\). CLP surgery significantly increased the serum concentrations of cTn-I and

![Fig. 3](image-url)  
**Fig. 3** Effects of sodium tanshinone II A sulphonate (STS) on serum biomarkers levels in rats with sepsis. STS at doses of 5 and 15 mg·kg\(^{-1}\) or equal volume of normal saline were i.v. administered immediately post-CLP or sham surgery. Drug/saline administration was repeated every 24 h for 3 days. Blood samples were collected at 24, 48, and 72 h post-CLP or sham surgery. Serum levels of biomarkers are shown as means ± SD, \(n = 10\). †\(P < 0.05\), ††\(P < 0.01\) vs sham group; *\(P < 0.05\), **\(P < 0.01\) vs CLP group
cTn-T, which indicated a severe myocardial dysfunction. The low dose STS treatment had limited attenuating effects on cTn-I and cTn-T, and only could significantly reduce the cTn-T level at 48 h ($P < 0.05$), and the cTn-I levels were still significantly higher than that in the sham group ($P < 0.05$ at 24 and 48 h). The high dose STS treatment significantly decreased the serum concentration of cTn-T at the three time points ($P < 0.05$), and also remarkably lowered the cTn-I level at 72 h ($P < 0.05$). The serum concentration of cTn-I and cTn-T in the high dose STS group approached to the levels seen in the sham group. The beneficial effects of high dose STS on cTn-I and cTn-T levels were better than that of low dose STS at 48 and 72 h after surgery.

BNP is another diagnostic marker for myocardial dysfunction which is also a useful predictor of mortality in sepsis [29]. CLP surgery increased the BNP level at 24, 48, and 72 h ($P < 0.01$). Although low dose STS reduced the BNP serum concentration during the whole experiment, and even significantly decreased the BNP level at 24 h ($P < 0.01$), compared with the CLP group, the BNP level at all the time points were still higher than that in the sham group. On the contrary, compared with CLP group, high dose STS treatment remarkably decreased the BNP level ($P < 0.01$ at 24 h, $P < 0.05$ at 48 and 72 h). No significant differences in BNP levels were observed between the sham group and the STS high dose group. Alterations of cTn-I level and BNP level in STS treatment groups suggested that STS might lessen myocardial dysfunction in the CLP rats. Taking the effects of STS on cardiac function into consideration, STS could actually attenuate cardiac dysfunction in CLP rats.

**Effects of STS on myocardial inflammatory cytokines**

CLP surgery significantly increased the levels of TNF-$\alpha$, IL-1$\beta$, IL-6 IL-10 and HMGB1 at each time point, indicating severe inflammation in myocardium (Fig. 4, $P < 0.01$ or $P < 0.05$). High dose STS treatment began to remarkably decrease the TNF-$\alpha$ concentration in myocardium at 48 h (Fig. 4A, $P < 0.05$), and the therapeutic effect lasted to 72 h ($P < 0.01$). Both low dose and high dose STS could decrease the myocardial IL-1$\beta$ level. The effect of STS on IL-6 secretion was similar to that on IL-1$\beta$ secretion, except for a significant decreasing effect of high dose STS observed at 48 h (Fig. 4C, $P < 0.05$) and 72 h ($P < 0.05$). STS treatment had no effect on IL-10 secretion (Fig. 4D). The myocardial IL-10 levels in the CLP group and treatment groups were still at the same level after drug administration. Compared with the CLP group, high dose STS could markedly decrease the myocardial HMGB1 level in a continuous mode throughout the experiment (Fig. 4E, $P < 0.05$). The results from this experiment revealed that STS could decrease the concentrations of proinflammatory cytokines (TNF-$\alpha$, IL-1$\beta$ and IL-6) and HMGB1 in myocardium, which was beneficial for the control of myocardial inflammation and could lessen the degree of damage to the myocardium. It was also noteworthy that STS treatment had little influence on IL-10 levels, indicating that it might have limited effects on proinflammatory cytokine feedback system.

![Fig. 4](image-url)Effects of sodium tanshinone II A sulphonate (STS) on myocardial inflammatory cytokine levels in rats with sepsis. STS at doses of 5 and 15 mg.kg$^{-1}$ or equal volume of normal saline were i.v. administered immediately post-CLP or sham surgery. Drug/saline administration was repeated every 24 h for 3 days. Heart samples were collected at 24, 48, and 72 h post-CLP or sham surgery. Myocardial homogenates were prepared for measurement of inflammatory cytokine levels. Cytokine levels are shown as means ± SD, n=10. † $P < 0.05$, †† $P < 0.01$ vs sham group; * $P < 0.05$, ** $P < 0.01$ vs CLP group.
Effects of STS on myocardial damage

The results of histopathological evaluation are shown in Fig. 5, and the inflammation scores are shown in Fig. 5M. In the sham group, minimal histological changes could be found, such as few acidophilic degenerations, and there was no inflammation associated with sham surgery at the three time points (Figs. 5A, 5E, and 5I). The inflammation almost increased to grade 3 at the 24 h after CLP surgery (Fig. 5M).

Myocardial interstitial edema and inflammatory infiltration were obviously observed in the CLP group (Fig. 5B). The longer the postoperative time, the severer the inflammation in the CLP group. The inflammation increased to about grade 4 at 72 h (Fig. 5M), and necrosis was found (Figs. 5F and 5J). Low dose STS could attenuate the myocardial damage, which was represented as lower inflammatory scores and absence of necrosis.

However, the therapeutic effect of low dose STS was limited, inflammatory infiltration and myocardial interstitial edema still could be found (Figs. 5C, 5G, and 5K). Compared with the CLP group, high dose STS significantly decreased the inflammation scores at 48 and 72 h (Fig. 5M, \(P < 0.01\)). No obvious inflammatory infiltration and necrosis were found, and only mild interstitial edema and few acidophilic degenerations were noted (Figs. 5D, 5H, and 5L).

Effects of STS on survival rate of rats with CLP-induced sepsis

No rat died in sham group throughout the survival experiment. On the contrary, all the rats treated with normal saline alone in CLP group died within 8 days after CLP surgery (Fig. 6). However, compared with the CLP group, 60% of rats in low dose STS group and 50% of rats in high dose STS group died by Day 8 after surgery, and the survival rate of the high dose STS group was significantly higher than that of CLP group \((P < 0.05)\). As the experiment went on, the rats from the two STS treatment groups died, and the survival rate fell to 20% and 30% by Day 18, respectively (Fig. 6). The difference in overall survival rate between CLP group and high dose group was significant \((P < 0.05)\).

Discussion

Sepsis resulting from bacterial, viral, fungal and parasitic
infections triggers the release of various inflammatory mediators and induces cellular and organic dysfunction in the affected host [30]. There are three phases in the pathogenesis of sepsis: (1) infection of pathogenic microorganism and release of toxins; (2) release of cytokines; and (3) destructive effects of excessive specific cytokines [30]. CRP is a sensitive systemic marker of inflammation and tissue damage, and PCT increases markedly in serum of humans and animals with sepsis. It has been shown that PCT serum level is associated with the mortality in sepsis, and it can be a useful prediction marker in sepsis. According to 2012 international guidelines for management of severe sepsis and septic shock, plasma CRP coupled with the plasma PCT is a considerable inflammatory marker in sepsis. According to 2012 international guidelines for management of severe sepsis and septic shock, plasma CRP coupled with the plasma PCT is a considerable inflammatory variable in the diagnosis of sepsis [31]. In the present study, the serum levels of PCT and CRP in the CLP group were about two standard deviation (SD) above the level of the sham group, and the two indicators were significantly decreased after STS treatment. It indicated that the STS could relieve inflammatory reactions in septic rats.

Cardiac dysfunction is a well-recognized complication of severe sepsis and septic shock [32]. It is also a major contributor to morbidity and mortality in patients with sepsis. Cardiac dysfunction is characterized by reduced contractility, decrease in ejection fraction, and ventricular dilatation. The LVSP and the dp/dt max are two important parameters of the left ventricular systolic function, while the LVEDP and the maximal rate of −dp/dt max predict the left ventricular diastolic function. In the present study, sepsis caused an increase in LVEDP and decreases in LVSP, −dp/dt max, MAP and HR in the CLP group, suggesting an early stage of heart failure. STS could significantly attenuate the cardiac dysfunction in rats with sepsis. In addition to these macroscopical changes in hemodynamic parameters, decreases in serum biomarkers, such as cTn-I, cTn-T and BNP, were also monitored after treatment with STS. It has been shown that cTn-I and cTn-T are expressed as cardio-specific isoforms, and they are predictors for the detection of myocardial damage [33]. BNP, secreted from atria and ventricles in response to volume load and myocardial wall stress, is another sensitive indicator for the diagnosis of cardiac dysfunction, especially for left ventricular dysfunction [34]. The results from the present study indicated that the STS could actually attenuate the cardiac dysfunction.

A critical question was then raised: what mechanism resulted in STS attenuating sepsis-induced cardiac dysfunction? As sepsis is associated with the abnormal host immune function in response to invading pathogens and the “cytokine storm” is thought to be responsible for triggering the inflammation in sepsis, the effects of STS on various cytokines in myocardial tissue, including TNF-α, IL-1β, IL-6, IL-10 and HMGB1, were examined in the present study to reveal its mechanism on attenuating sepsis-induced cardiac dysfunction. Cytokines TNF-α and IL-1β are primarily secreted by macrophages and act as proinflammatory mediators. Both cytokines play a major role in initiation and regulation of inflammation and immunity response. TNF-α and IL-1β are “proximal” cytokines, which are secreted early in sepsis [32]. Previous researches have indicated that high concentration of TNF-α induces myocardial cell apoptosis [35], has a negative inotropic effect on isolated cardiac myocytes, and decreases myofilament sensitivity to Ca2+ [36], and that IL-1β has also been shown to increase the activity of Na+–Ca2+ exchanger [32]. Cardiomyocyte TNF-α and IL-1β secretion is responsible for myocardial depression and haemodynamic abnormalities [32]. Furthermore, these two proximal cytokines stimulate the production of IL-6, a “distal” cytokines. Unlike IL-1β and TNF-α, IL-6 does not induce cytokine expression and its main effects are to act in synergy with IL-1β and TNF-α to augment the response of immune cells to other cytokines. In addition, IL-6 can activate the coagulation system and function as a pyrogen. Inhibition of TNF-α and IL-1β expression results in a decrease in IL-6 production. In the present study, STS, particularly at high dose, significantly decreased the myocardial TNF-α and IL-6 levels. However, it only decreased the myocardial IL-1β level slightly, and no significant differences were observed between treatment groups and CLP group. The alleviating effect of STS on IL-6 could be mainly due to its efficient decrease in TNF-α.

HMGB1 is another important proinflammatory cytokine in the pathophysiology of sepsis. HMGB1 protein is originally described as a nuclear DNA-binding protein in the gene expression and transcriptional regulation. It has been functionally characterized as an “danger signal” or “alarmin”, and is released from activated monocytes and macrophages, pi tuicytes, enterocytes, hepatocytes, and necrotic damaged cells [36]. Recent studies in mouse and rat models of sepsis have shown that HMGB1 can be secreted by dying or necrotic cardiomyocytes. Even viable cardiac myocytes have the ability of HMGB1 secretion [37]. HMGB1 modulates the membrane calcium influx and thus decreases calcium availability in cardiac myocytes. It acts as a myocardial depressant factor and has an obvious negative impact on myocardial contractile function, which is mainly manifested as decreasing LVDP, left ventricular (LV) +dp/dt max and absolute value of LV −dp/dt max, and increasing LV end-diastolic pressure [38]. Furthermore, HMGB1 plays a critical role in mediating organ damage in sepsis [37, 39], and HMGB1 antagonists exhibit protective roles against lethal sepsis [1]. In the present study, high dose STS significantly inhibited the myocardial HMGB1 secretion, which was beneficial to enhance the cardiac function and protecting rats with sepsis from death. As HMGB1 secretion can be affected by endotoxin or various proinflammatory cytokines, such as TNF-α and IL-1β, the suppressive effect of STS on HMGB1 secretion might be partially attributed to its excellent inhibition of myocardial TNF-α. In addition, unlike steroidal and nonsteroidal anti-inflammatory drugs, steroid-like STS dose-dependently attenuated endotoxin-induced HMGB1 secretion, which might also make a contribution to alleviating myocardial HMGB1 level in rats with sepsis.


Conclusion

In conclusion, STS treatment could inhibit overproduction of proinflammatory cytokines, including TNF-α, IL-6 and HMGB1, alleviating inflammatory reactions and enhancing cardiac function. Therefore, STS treatment could improve the survival rate of rats with CLP-induced sepsis. The findings suggested that STS may be a useful candidate drug for the treatment of septic myocardial dysfunction.

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Abbreviations

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<tr>
<th>CRP</th>
<th>C-reactive protein</th>
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<tr>
<td>PCT</td>
<td>Procalcitonin</td>
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<tr>
<td>BNP</td>
<td>Brain natriuretic peptide</td>
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<tr>
<td>cTn-I</td>
<td>Cardiac troponin I</td>
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<tr>
<td>cTn-T</td>
<td>Cardiac troponin T</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
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<td>IL-6</td>
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<tr>
<td>HMGB1</td>
<td>High mobility group protein B1</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule 1</td>
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<td>VCAM-1</td>
<td>Vascular cell adhesion molecule 1</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharides</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>LVSP</td>
<td>Ventricular systolic pressure</td>
</tr>
<tr>
<td>LVEDP</td>
<td>Left ventricular end-diastolic pressure</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial blood pressure</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>dp/dtmax</td>
<td>Maximal rate of rise of left ventricular pressure</td>
</tr>
<tr>
<td>-dp/dtmax</td>
<td>Decline of the left ventricular pressure</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin-eosin</td>
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</table>

References

[19] Yang L, Zou XJ, Gao X, et al. Sodium tanshinone IIA sulfoxonate attenuates angiotensin II-induced collagen type I ex-


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