Original Article

Shenfu injection attenuates cardiac dysfunction and inhibits apoptosis in septic mice

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Background: Cardiac dysfunction frequently occurs after sepsis and septic shock and contributes to multiple organ failure. Shenfu injection, a well-known Chinese medicine formulation, has recently been demonstrated to possess potential cardio-protective effects, although its effect and mechanism in sepsis-induced myocardial dysfunction remains unclear.

Methods: To investigate whether Shenfu injection could alleviate myocardial injury and improve cardiac function, a model of sepsis was induced by cecal ligation and puncture (CLP) surgery in C57BL/6 mice. Cardiac function, inflammatory factors, and apoptosis were evaluated in the myocardium.

Results: Our results showed that the survival and left ventricular function markedly declined when exposed to CLP compared with the sham procedure. In contrast, Shenfu injection lowered mortality and prevented CLP from triggering left ventricular dysfunction. Moreover, mice treated with Shenfu injection exhibited noticeably decreased myocardial inflammatory cytokines [tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and monocytechemoattractantprotein-1 (MCP-1)] and reduced apoptotic myocardiocytes death compared with mice that had CLP. The activation of myocardial NF-κB detected in septic mice was suppressed by the presence of Shenfu injection, as evidenced by decreased nuclear translocation of the NF-κB p65 subunit. Moreover, Shenfu injection also prevented sepsis-caused decreases in myocardial phospho-Akt and phospho-GSK-3β in septic mice.

Conclusions: These data suggest that administration of Shenfu injection attenuates cardiac dysfunction, protects against inflammatory injury, and reduces apoptosis of myocardiocytes during sepsis by activating the AKT/GSK-3β pathway.

Keywords: Sepsis-induced cardiac dysfunction; inflammation; apoptosis; Shenfu injection

Submitted Jan 24, 2022. Accepted for publication May 20, 2022.
doi: 10.21037/atm-22-836
View this article at: https://dx.doi.org/10.21037/atm-22-836

Introduction

Sepsis is a common and critical clinical syndrome that often has a poor prognosis (1). Severe sepsis can result in hemodynamic abnormalities and cardiac dysfunction (2). Despite extensive studies and investigation, the pathogenetic mechanisms that mediate sepsis-induced cardiac dysfunction remain elusive. A growing body of evidence suggests that the pathophysiological process responsible for cardiac dysfunction during sepsis involves the host immune response, myocardial inflammatory injury, and apoptosis (3). Myocardial dysfunction as a result of sepsis has high morbidity and mortality rates, therefore effective strategies to attenuate or prevent sepsis-induced cardiac dysfunction are urgently needed.

Chinese medicines that have been used for thousands of years have been shown to benefit patients with sepsis and other infection diseases (4). In particular, Shenfu injection is a potent compound that has received a great deal of attention due to its multiple anti-inflammatory, anti-apoptosis, anti-oxidant, and innate immunity modulating effects (5,6). These pharmacological effects may be ascribed to the active components of this compound, which mainly consist of *ginsenosides* and *aconite alkaloids* (7). Experiments *in vivo* have established that treatment with Shenfu injection dramatically decreases the expression of tumor necrosis factor-α (TNF-α) and increases the Bcl-2/Bax ratio in a murine model of cecal ligation and puncture (CLP)-induced sepsis (8). In a multicenter, randomized, controlled trial consisting of patients with septic shock, Shenfu injection was demonstrated to improve the hemodynamic parameters and 7-day survival in those severely ill patients with lactate levels >4.5 mmol/L (9). Another prospective, randomized, controlled clinical study enrolled 1,022 patients with return of spontaneous circulation after cardiac rest. Patients treated with Shenfu injection had a significantly greater 28-day survival rate (42.7%) than the control group (30.1%) (10). It is possible, therefore, that administration of Shenfu injection could result in the improvement of cardiac dysfunction induced by sepsis. In the present study, we assessed sepsis-induced cardiac injury and dysfunction in patients who were or were not treated with Shenfu injection. We for the first time explained the mechanism of Shenfu injection in alleviating sepsis-induced cardiac dysfunction from the perspective of inhibiting the expression of myocardial inflammatory cytokines, myocardiocytes apoptosis, and myocardial activated Akt/GSK-3β in septic mice. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-836/rc).

Methods

Animals

Age- and weight-matched male C57BL/6 mice were obtained from the Medical Experimental Animal Center of Guangdong (Guangzhou, China). A protocol was prepared before the study without registration. The protocol used in this paper was approved by the Animal Care and Use Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (No. B2016-541-01), in compliance with the Care and Use of Laboratory Animals national guidelines for the care and use of animals.

CLP septic procedure and design

The experimental mice underwent CLP procedure to induce sepsis in mice (11). Mice aged 10 weeks and weighing 30 g were anesthetized with isoflurane (Rhodia UK Ltd., Avonmouth, UK) and ventilated. Mice were divided into 5 groups of 20 mice each: control, sham-surgically operation (sham), CLP operation+ normal saline (NS), CLP operation+ low concentration of Shenfu injection (SF-L), CLP operation+ high concentration of Shenfu injection (SF-H). An incision was made in the abdomen, and then the cecum was isolated carefully. After ligation below the ileocecal junction with a 4-0 suture, the cecum was punctured twice with an 18-gauge needle. The abdominal wall was closed after the cecum was returned to the abdominal cavity. For sham-surgically operated mice, the cecum was isolated in the same procedure but without ligation and puncture. The mice that did not receive any procedures were considered normal controls. For those subjected to the CLP procedure, the mice were randomly divided into 3 groups. One hour prior to surgery, 2 group was treated with Shenfu injection at 10 mg/kg body weight (SF-L) and 40 mg/kg body weight SF-H), and the other was given 0.9% normal saline (NS) by intraperitoneal injection. This dose of Shenfu injection was found to be cardio-protective in the sepsis rodent model (8). The quality of Shenfu injection was strictly controlled according to the criterion of the China Ministry of Public Health

(official approval code: certification number 220043117; No.110804, China). At 72 hours post-procedure, the hearts of all experimental mice were isolated under anesthesia after echocardiographic assessments. The myocardium was frozen in liquid nitrogen or fixed in formalin for further analysis.

Echocardiography assessment

Two hours before or 72 hours after the CLP procedure, echocardiography was used to evaluate cardiac function (12). Mice were anesthetized with 5% isoflurane (Rhodia UK Ltd.) mixed with oxygen. The two-dimensional (2-D) image was obtained using a 25-MHz RMV-707B transducer connected to the Vevo 770 imaging system (Visual Sonics, Toronto, Canada). A 2-D short-axis view of the left ventricle (LV) was obtained at the level of the papillary muscles, and 2-D M-mode tracings were documented. The LV end-diastolic dimension (LVEDD) and LV end-systolic dimension (LVESD) were measured using an online analyzing system (Visual Sonics, Toronto, Canada) to calculate the LV fractional shortening (LVFS). All measurements were made according to the guidance from the American Society of Echocardiography.

Quantitative real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from the mice myocardium using the RNA spin Mini Kit (GE Healthcare, Buckinghamshire, UK). Myocardial TNF-α, interleukin (IL)-1β, IL-6, and mo nocytechemoattractantprotein-1 (MCP-1) RNA levels were measured using quantitative RT-PCR (Applied Biosystems). Primer pairs used are shown in Table S1. Relative levels of gene expression were normalized to β-actin expression levels (13).

Western blot analysis

Myocardial protein samples were loaded and separated with 10–15% polyacrylamide gel and transferred to polyvinylidene difluoride membranes. The membranes were probed overnight with anti-caspase3-, anti-Bcl-2-, anti-BAX-, anti-NF-κB-p65-, anti-phosphor-AKT-, anti-phospho-GSK-3β-, and anti-tubulin-antibody at 4 ℃. The proteins were identified using fitting peroxidase-labeled secondary antibodies and a chemiluminescence substrate at room temperature.

Terminal deoxynucleotidyl transferase dUTP nick end labeling staining assay

The cleavage of DNA during apoptosis was determined with the Fluorescein FragEL kit (Oncogene Research Products, Boston, USA) and performed according to the manufacturer's instructions. The total number of TUNEL-positive myocardiocytes was determined in 10 randomly selected fields for each heart (14).

Enzyme-linked immunosorbent assay measurements

Whole blood was harvested 3 days after the mice underwent the CLP or sham procedure. The concentration of TNF-α, IL-1β, IL-6, and MCP-1 were evaluated in the serum using mouse multiple proinflammatory cytokines assay kits (R&D Systems Inc, Minneapolis, USA) (15).

Statistical analysis

All data are described as means ± standard error of mean throughout. Significant differences between compared groups were determined by using the Student's *t*-test or one-way analysis of variance (ANOVA) using SPSS 12.0 (IBM, Armonk, NY, USA). A value of $P<0.05$ was considered significant.

Results

Shenfu injection improved survival of mice after CLP

The overall survival rate of the Shenfu injection group was higher than that of the CLP only group at 72 hours after procedure ($P<0.05$). Moreover, no mice died in the sham groups treated with 0.9% NS or the normal control group (*Figure 1*).

Shenfu injection attenuates septic myocardial dysfunction

The cardiac function of all experimental mice was evaluated before and 3 days after the CLP or sham procedure. There were no detectable differences in echocardiographic structure and function, including LV ejection fraction (LVEF) and LVFS, between the control and sham group (*Figure 2*; Table S2).

At 72 hours after the CLP procedure, LVEF, LVFS, LV end-systolic pressure (LVESP), and dP/dt were markedly impaired in the CLP-challenged mice compared with mice

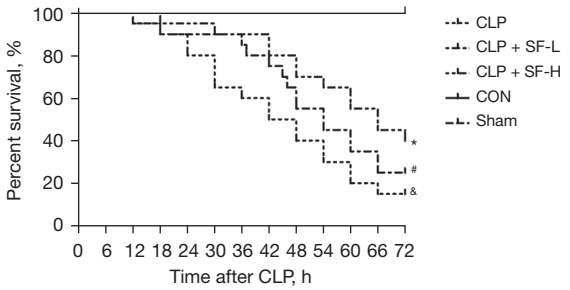

Figure 1 SF enhances septic mice survival induced by CLP. Kaplan-Meier curves of survival in mice from the control (CON), sham (Sham), CLP, CLP + 10 mg/kg SF (CLP + SF-L), and CLP + 40 mg/kg SF (CLP + SF-H) groups. Time is expressed in hours after the operation (n=20 each group). $^{\&}$, P<0.05 *vs.* sham; $^{\#}$, P<0.05 *vs.* CLP; *, P<0.05 *vs.* treated with SF-L. SF, Shenfu injection; CLP, cecal ligation and puncture.

in the control or sham groups (all P<0.05). Administration of Shenfu injection attenuates the CLP-induced decreases in LVEF, LVFS, dP/dt, and LVESP (P<0.05).

Similarly, mice in the CLP group had a higher LVESD and a marked decrease in LVEDD compared with control hearts (P<0.05), indicating a considerable reduction in cardiac contractile function in the septic mice. However, cardiac dysfunction induced by CLP was attenuated in Shenfu injection-treated mice as evidenced by a lower increase in LVESD (P<0.05) and only a slight decrease in fraction shortening (FS) %, indicating that Shenfu injection prevented CLP-induced myocardial dysfunction. Significantly, the administration of a high-dose Shenfu injection (40 mg/kg) largely improved LV systolic function compared with a lower dose (10 mg/kg).

Shenfu injection inhibits CLP-induced myocardial production of inflammatory cytokines

Previous research shows that cardiac dysfunction is a critical complication in the setting of sepsis and that the inflammatory signaling could be the major mediator that leads to this pathological process (16). As presented in *Figure 3*, the levels of TNF-α, IL-1β, IL-6, and MCP-1 in myocardium of septic mice were assessed by PCR and enzyme-linked immunosorbent assay (ELISA) analysis. The myocardial TNF-α, IL-1β, IL-6, and MCP-1 mRNA were dramatically increased in septic mice after the CLP procedure compared to those of the control and sham groups (*Figure 3*; P<0.05). All these increased mRNA levels of inflammatory cytokines were attenuated in septic mice treated with Shenfu injection (*Figure 3*; P<0.05). This pattern of mRNA expression change was also seen at the protein level, indicating that administration of Shenfu injection suppressed the inflammatory responses in septic hearts (*Figure 3*).

Shenfu injection inhibits apoptosis in the myocardium of septic mice

Myocardial apoptosis has been reported to play a crucial role in sepsis-induced multiple organ dysfunction (17,18). Therefore, we further tested the effect of Shenfu injection on apoptosis of myocardiocytes in septic mice. Compared with CLP mice, both cleaved caspase-3 and Bcl2-associated X (Bax) were reduced in septic mice treated with Shenfu injection (*Figure 4*). Anti-apoptotic factors for Bcl-2 were also examined using an immunoblot assay. CLP exposure reduced myocardial expression of Bcl-2. Conversely, treatment with Shenfu injection preserved myocardial Bcl-2, suggesting that Shenfu injection at least partially reverses the sepsis-induced imbalance of pro-apoptotic and anti-apoptotic pathophysiological processes.

Apoptotic myocardiocytes were also stained using TUNEL assay (*Figure 4B*). A significant increase in TUNEL-positive cells was observed in the cardiac sections from septic mice compared with the control group. In contrast, after treatment with Shenfu injection, TUNEL-positive fewer myocardiocytes were found in mice after CLP.

Shenfu injection blocks CLP-induced NF-κB activation in septic myocardium

Existing research has demonstrated that NF-κB activation mediates inflammatory signaling and contributes to the pathogenesis of cardiac injury and dysfunction in response to sepsis (19). To examine the mechanisms by which Shenfu injection prevents inflammatory injury, we examined p65 translocation into the nucleus using an immunoblot. Translocation of the NF-κB p65 subunit from the cytosol into the nucleus was evident in myocardium during sepsis. However, treatment with Shenfu injection significantly reduced this nuclear translocation (*Figure 5*).

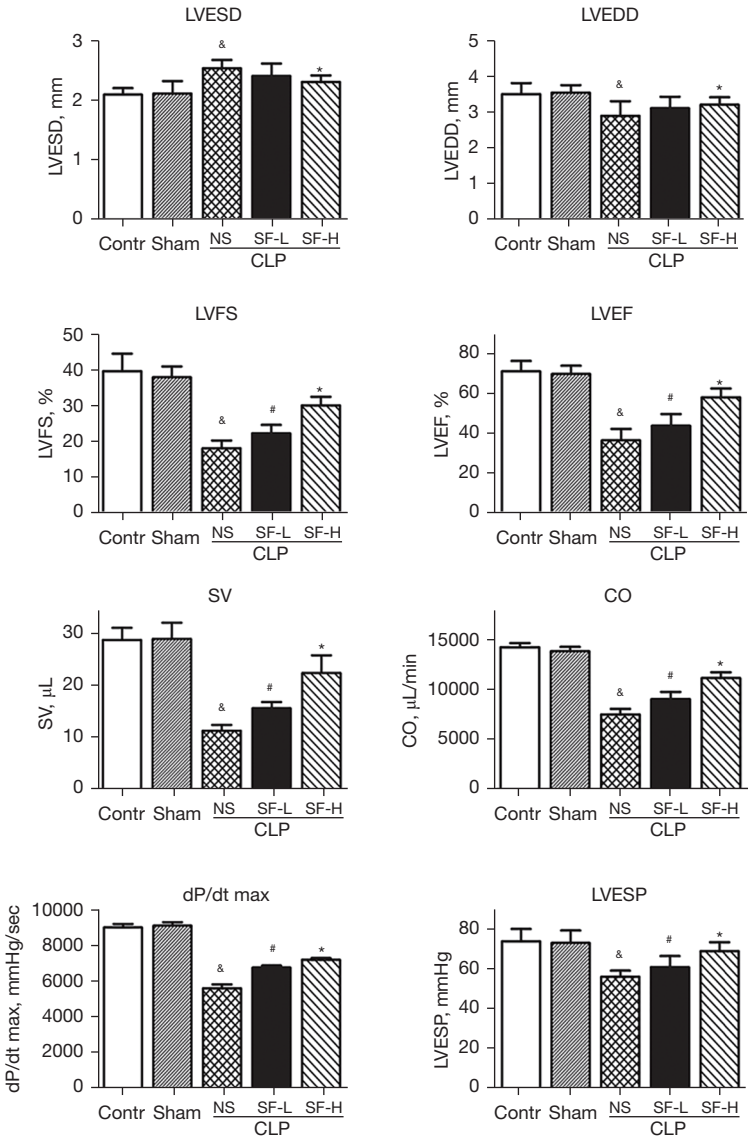

Figure 2 SF improved cardiac function in in septic mice. Quantitative analysis of the LV dimension and systolic/diastolic function in septic mice exposed to SF or NS (n=10). Data are presented as mean ± SD of three independent experiments. [&], P<0.05 *vs.* sham; [#], P<0.05 *vs.* CLP; *, P<0.05 *vs.* treated with SF-L. CLP, cecal ligation and puncture; SF, Shenfu injection; SF-L, 10 mg/kg SF; SF-H, CLP + 40 mg/kg SF; NS, normal saline; LVESD, left ventricular end-systolic diameter; LVEDD, left ventricular end-diastolic diameter; LVFS, left ventricular fractional shortening; LVEF, left ventricular ejection fraction; SV, stroke volume; CO, cardiac output; LVESP, left ventricular end-systolic pressure; LV, left ventricle.

Shenfu injection prevented the downregulation in myocardial phospho-Akt/GSK-3β in sepsis

Previous data showed that the phosphorylation of Akt attenuates myocardiocytes apoptosis (20). Activation of the PI3K/Akt pathway increases long-term survival in the animal model of sepsis in an Akt-dependent manner (21). To assess the effect of Shenfu injection on the activation of Akt in the animal sepsis model, we examined the levels of phospho-Akt in the myocardial homogenates. As shown in *Figure 5*, the expression of myocardial phospho-Akt was significantly decreased in mice from the CLP only group compared with the control and sham groups. In contrast, treatment with Shenfu injection prevented the reduction in phospho-Akt in CLP-induced septic mice (*Figure 5*). Furthermore, 40 mg/kg

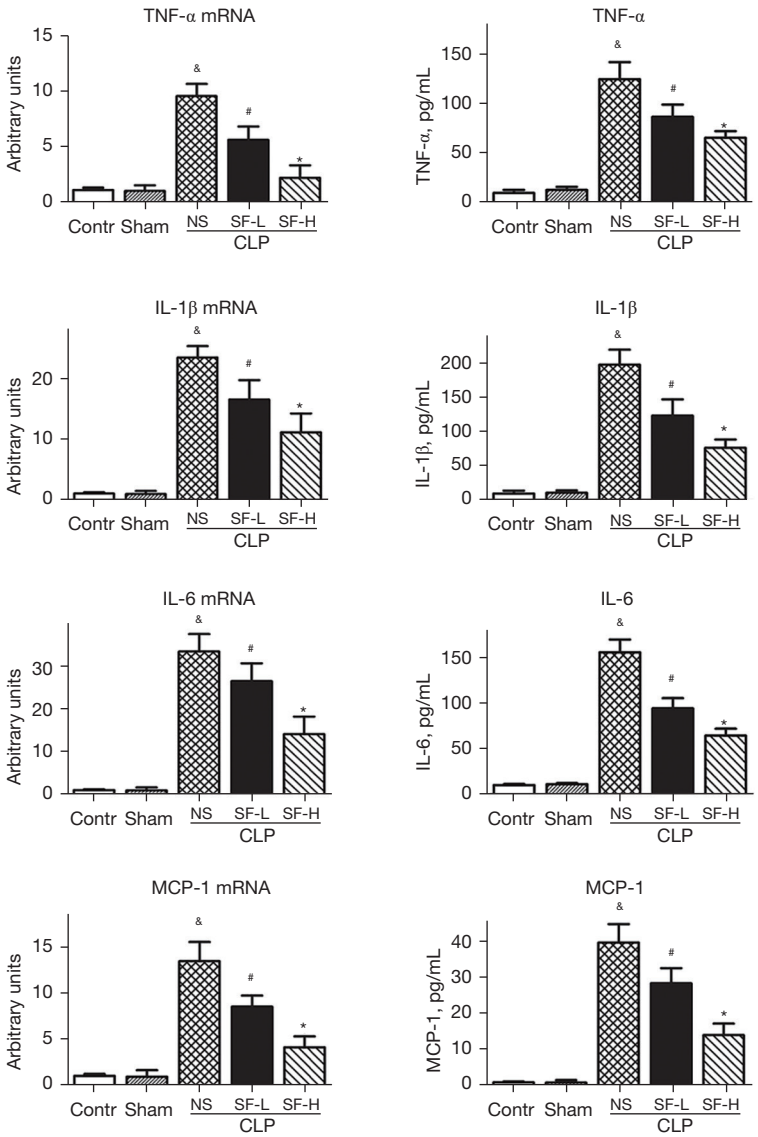

Figure 3 SF reduced myocardial inflammatory cytokine production in septic mice. Myocardial TNF-α, IL-1β, IL-6 and MCP-1 mRNA expression and protein levels in the NS- and SF-treated mice were assayed by quantitative real-time PCR or ELISA. Data are presented as mean ± SD of three independent experiments. [&], P<0.05 *vs.* sham; [#], P<0.05 *vs.* CLP; *, P<0.05 *vs.* treated with SF-L. SF, Shenfu injection; SF-L, 10 mg/kg SF; SF-H, CLP + 40 mg/kg SF; NS, normal saline; CLP, cecal ligation and puncture; PCR, polymerase chain reaction; TNF-α, tumor necrosis factor-α; IL, interleukin; MCP-1, monocytechemoattractantprotein-1.

of Shenfu injection induced a level of phospho-Akt in the myocardium that was significantly higher than 10 mg/kg.

Additionally, we examined the levels of phosphorylation of GSK-3β, which existing research shows is a downstream mediator of the phospho-Akt in the myocardium (22). As shown in *Figure 5*, the levels of phospho-GSK-3β (Ser9) were dramatically decreased (64.7%) in mice subject to CLP when compared with the control and sham one. However, administration of Shenfu injection increased the myocardial phospho-GSK-3β levels in mice subjected to CLP compared to the mice that were treated with 0.9% NS after the CLP procedure (*Figure 5*).

Discussion

It is now generally accepted that cardiac dysfunction during sepsis

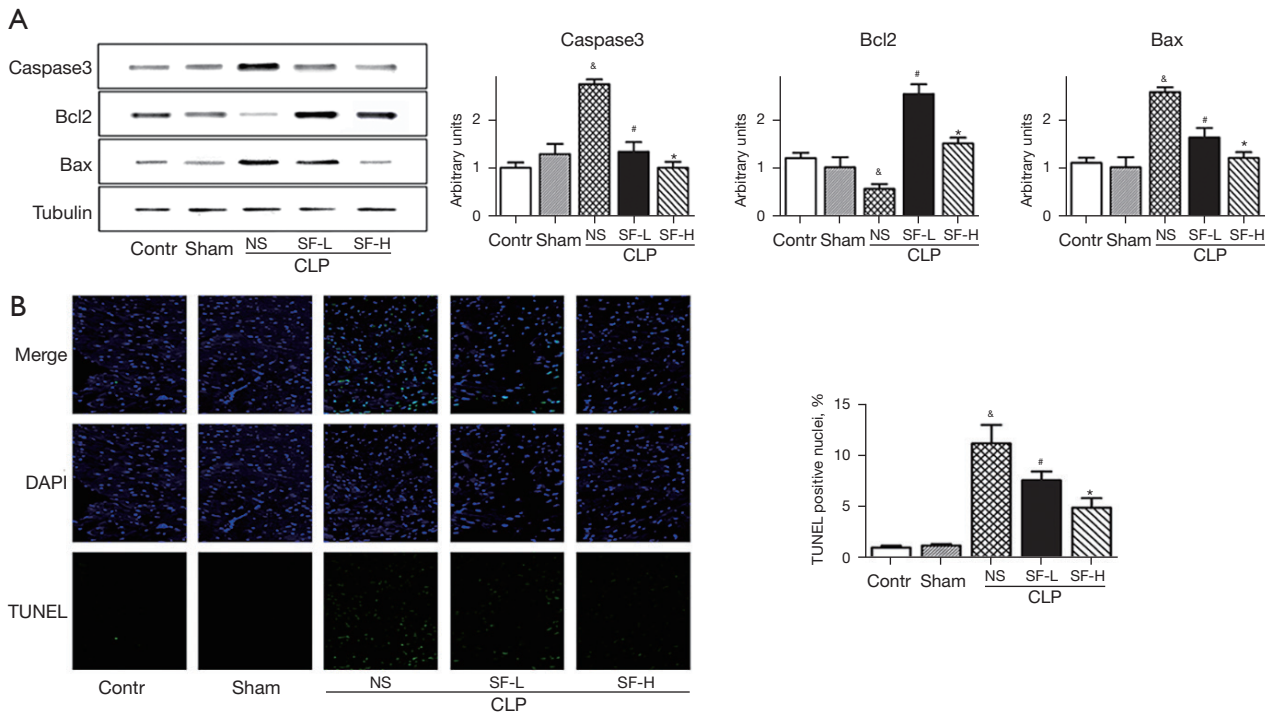

Figure 4 SF inhibited cardiomyocytes apoptosis in septic mice. (A) Apoptotic protein cleaved caspase3, Bax, and anti-apoptotic protein Bcl2 levels were analyzed by immunoblotting. Data were normalized using tubulin. (B) Representative TUNEL staining of the left-ventricular myocardium section. Blue, DAPI stained nuclei. Original magnification, 400×; scale bar, 100 μm. Quantification of TUNEL positive cells was shown in right panel. &, P<0.05 *vs.* sham; #, P<0.05 *vs.* CLP; *, P<0.05 *vs.* treated with SF-L. SF, Shenfu injection; SF-L, 10 mg/kg SF; SF-H, CLP + 40 mg/kg SF; CLP, cecal ligation and puncture; NS, normal saline; TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labeling.

may be a result of the interaction of many factors, including inflammation, oxidative stress, metabolism, mitochondrial dysfunction and neuroimmunomodulation (23). During sepsis, various pathogens activate Toll-like receptors (TLR) (especially TLR-4) through pathogen-related molecular patterns (PAMPs) such as LPS, which promote the infiltration of inflammatory cells in myocardial tissue and cause myocardial inhibition (24). A variety of TLRs signal through the myeloid differentiation factor 88 (MyD88)-dependent pathway and activate multiple signaling pathways including NF-κB and extracellular signal-regulated kinases 1/2 (ERK1/2), which in turn induce the production of multiple proinflammatory cytokines, including IL-1β, IL-6 and TNF-α (25,26). These factors amplify the inflammatory cascade in the early stages of sepsis. During sepsis-induced myocardial injury, the effects of inflammation on mitochondrial function lead to an increase in the production of reactive oxygen species (ROS) (27). A large number of ROS metabolites lead to myocardial cell death by inducing myocardial cell damage and structural dysfunction of the myocardial microcirculation system. Initiation and execution of these processes are regulated by the BCL-2 and caspase families of proteins. Cytochrome c then bind Apaf-1 forming the apoptosome and activating caspase-9. Once active, caspase-9 can directly cleave and activate caspase-3 and caspase-7 and induce myocardial cell apoptosis (28).

The main active components of Shenfu injection are ginsenosides and higenamine. Meta-analysis suggest that Shenfu injection may benefit patients with heart failure or septic shock (29,30). Modern pharmacological research shows that ginsenosides can improve ischemic myocardium metabolism, protect myocardial ultrastructure, and reduce Ca^{2+} overload. Animal studies have found that ginsenoside Rg1 may exert its effect in reducing LPS-induced inflammation and apoptosis in NRCMs and sepsis mice by blocking the TLR4/NF-κB/NLRP3 pathway (31). Another study suggested that G-Rg1 may play the same

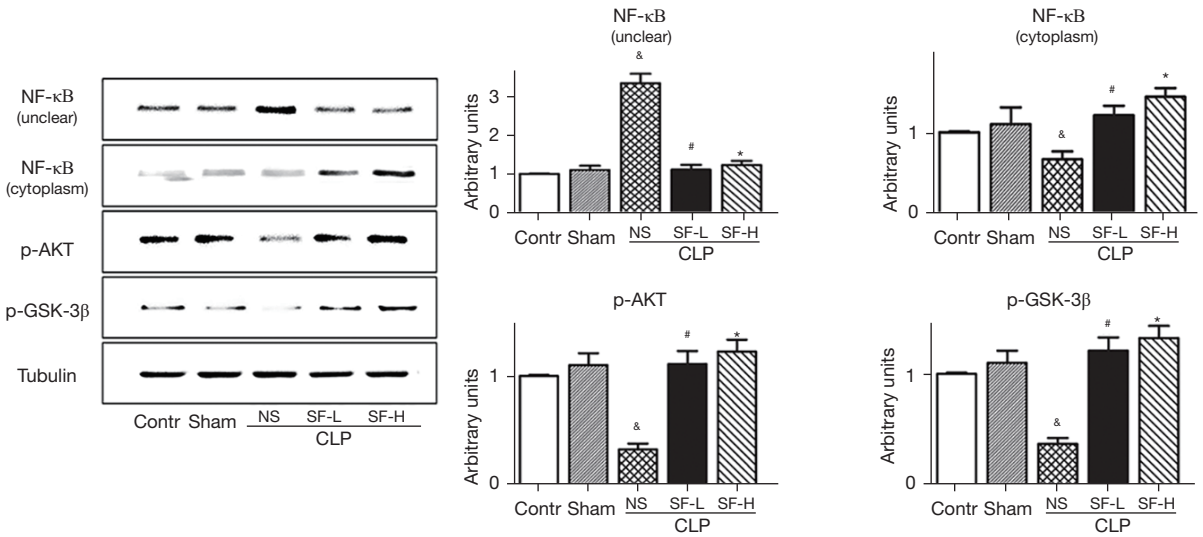

Figure 5 SF regulates NF-κB and AKT/GSK-3β in septic mice. Myocardial cytoplasm and nuclear NF-κB levels and phospho-AKT/GSK-3β were analyzed by immunoblotting. Data were normalized using tubulin. Quantification of densitometry of these proteins was shown in right panel. [&], P<0.05 *vs.* sham; [#], P<0.05 *vs.* CLP; *, P<0.05 *vs.* CLP. SF, Shenfu injection; SF-L, 10 mg/kg SF; SF-H, CLP + 40 mg/kg SF; CLP, cecal ligation and puncture; NS, normal saline.

role by activating the Akt/GSK-3β pathway through the P2X7 receptor (32). It has also been demonstrated in *in vitro* and *in vivo* sepsis models that G-Rg3 can up-regulate the autophagy-related proteins and activate AMPK signal pathway (33). Higenamine can enhance heart contractility, improve coronary circulation, and decrease the effect of acute myocardial ischemia (34). The heart protection and therapeutic effects of higenamine on heart disease are related to regulating LKB1/AMPKα/Sirt1, mediating the β2-AR/PI3K/AKT cascade, suppressing TGF-β1/Smad signaling, and targeting ASK1/MAPK (ERK, P38)/NF-κB signaling pathway (35).

A significant finding of this study is that administration of Shenfu injection improved the survival rates and mitigated cardiac injury and dysfunction in CLP-induced septic mice. We also reported that Shenfu injection potently prevented the enhanced expression of myocardial TNF-α, IL-1β, IL-6, and MCP-1 and myocardiocytes apoptosis following the CLP procedure. The Shenfu injection-induced cardioprotection was correlated with the suppression of myocardial NF-κB p65 nuclear translocation and elevation of Akt/GSK-3β activity in septic mice. These results suggest that the mechanisms of Shenfu injection that attenuated sepsis-induced cardiac dysfunction were related to the regulation of myocardial Akt/GSK-3β, reduction of inflammatory cytokines expression, and apoptosis.

Previous data showed that treatment with Shenfu injection significantly increased the survival of human and septic animal models because the underlying mechanisms protected the function of the brain, lung, liver and kidney by attenuating ischemia reperfusion (36,37). Consequently, we postulated that administration of Shenfu injection could also improve cardiac function during sepsis. To evaluate our hypothesis, we assessed cardiac structure and function in mice subjected to the CLP procedure in the presence or absence of administration of Shenfu injection. We found that the LV function detected by echocardiography was depressed in the mice model of sepsis. However, cardiac function was maintained in Shenfu injection-treated septic mice.

Although the pathogenic mechanisms of cardiac dysfunction during sepsis remain unclear, existing research has confirmed that pro-inflammatory cytokines play a critical role in this process (1). A study has demonstrated that the exposure to pro-inflammatory cytokines could synergistically depress global cardiac function characterized by decreased LVFS and cardiac output during sepsis (38). Herein, we reported that the CLP procedure resulted in nuclear translocation of myocardial NF-κB and the subsequent transcription and expression of TNF-α, IL-1β, IL-6, and MCP-1. This result is consistent with previous reports that show that the septic shock model

is characterized by NF-κB activation and production of inflammatory cytokines in the myocardium (39). Our study showed that administration of Shenfu injection improved cardiac dysfunction by negatively regulating the myocardial proinflammatory cascade and reducing the expression of TNF-α, IL-1β, IL-6, and MCP-1 in the myocardium in an experimental sepsis model.

Recent studies have shown that apoptosis of myocardiocytes is a major contributor to cardiac dysfunction and morbidity and mortality during sepsis (40). Inhibition of apoptosis with specific pharmacological inhibitors significantly enhanced survival in the murine model of sepsis (18). Support for this concept can also be found in the previous reports which showed that overexpression of Akt in the lymphocytes demonstrated reduced apoptosis and improved the survival rates in septic mice (41). Herein, we reported that the apoptosis of myocardiocytes was meaningfully upregulated in the sepsis mice model. Treatment with Shenfu injection considerably reduced apoptosis of myocardiocytes and inhibited caspase-3 from being cleaved in the myocardium of the CLP-induced septic mice. Moreover, Shenfu injection prevented the downregulation of the level of Bcl-2 in septic mice. This evidence was supported by other observations that showed that the Shenfu injection significantly decreased neural apoptosis and enhanced Bcl-2 expression in gerbils following cerebral ischemic reperfusion (42).

Enhancement of the activity of the Akt pathway has been demonstrated to inhibit apoptosis and improve the function of myocardiocytes *in vitro* (43). In contrast, pharmacological blocking of Akt was found to induce apoptosis in septic rats (21). Herein, we demonstrated that Shenfu injection averted the reduction in the phosphorylation of Akt in the myocardium from the septic model. Moreover, treatment with Shenfu injection increased the level of myocardial phospho-GSK-3β. Previous data demonstrated that the phosphorylation of GSK-3β at Ser9 results in enzyme inactivity (44). Our data indicated that treatment with Shenfu injection initiates myocardial Akt and simultaneously inactivates GSK-3β. GSK-3β has been demonstrated to regulate the inflammatory response by differentially affecting the nuclear amounts of transcription factors in the NF-κB subunit p65. Specific inhibition of GSK-3β protected mice receiving endotoxin from the inflammatory cascade response and septic shock (45). The findings in this study demonstrate that Shenfu injection regulates the phosphorylation of Akt/GSK-3 activity that modulates the myocardial inflammatory response and apoptosis, which ultimately enhances the cardiac function in mice in response to sepsis.

Conclusions

The administration of Shenfu injection attenuated cardiac dysfunction and improved survival in septic mice that received the CLP procedure. The potential mechanisms by which Shenfu injection mitigated cardiac injury and dysfunction included the phosphorylation of Akt/GSK-3β, inhibition of nuclear translocation of NF-κB, and reduction of myocardiocytes apoptosis and inflammation. Future studies are needed to verify whether specific inhibition of the Akt/GSK-3β pathway would weaken or diminish the Shenfu injection-exerted cardioprotective effect and to verify the in-depth molecular mechanisms by which Shenfu injection suppresses apoptosis.

Acknowledgments

Funding: This work was financially supported by the Science Foundation of Guangdong Province (No. 2019A030313636) and the Department of Science and Technology of Guangdong Province (No. 2017B020247062 to Prof. Fengli Zhao).

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-836/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-836/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-836/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments were performed under a project license (No. B2016-541-01) granted by the Animal Care and Use Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine, in compliance with the Care and Use of

Laboratory Animals national guidelines for the care and use of animals.

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(English Language Editor: C. Mullens)

Cite this article as: Zhao L, Jin L, Luo Y, Wang L, Li Y, Xian S, Zhao F. Shenfu injection attenuates cardiac dysfunction and inhibits apoptosis in septic mice. Ann Transl Med 2022;10(10):597. doi: 10.21037/atm-22-836