Original Article
Resveratrol attenuates myocardial injury in septic rats via inhibition of the JAK2/STAT3 signaling pathway

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Abstract: Resveratrol (Res) is reported to exert anti-inflammatory and anti-oxidative properties. Sepsis-induced myocardial injury is tightly associated with inflammation. However, the mechanisms underlying the protective effect of Res remain unclear. The present study investigated the protective effect of Res on myocardial injury in septic rats and the role of the Janus Kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) signaling pathway. Res treatment was found to significantly inhibit the activation of JAK2 and STAT3 in myocardial tissue. It also attenuated the level of pro-inflammatory cytokines, including tumor necrosis factor-α and interleukin-1β in the serum and myocardial tissue. In addition, Res alleviated myocardial apoptosis. In conclusion, the results indicate that Res exhibits substantial therapeutic potential for the treatment of sepsis-induced myocardial injury via JAK2/STAT3 signaling inhibition.

Keywords: Resveratrol, sepsis, myocardial injury, JAK2/STAT signaling

Introduction

Sepsis is a leading cause of death in surgical intensive care unit patients, and the treatment of this disorder is still challenging [1]. During sepsis, detrimental stimuli, such as LPS, are able to trigger inflammatory responses. Various kinds of inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, macrophage migration inhibitory factor (MIF) [2], as well as high mobility group box 1 (HMGB1) [3] are involved in sepsis, contributing to the recruitment of leukocytes and subsequent organ damage [4]. For instance, sepsis can cause cardiac dysfunction [5]. Many reports have suggested that the importance of mitochondrial dysfunction in sepsis-induced cardiac dysfunction [6, 7]. Additionally, cell apoptosis and inflammatory responses are also involved in this disorder [8-10].

Resveratrol (Res), a polyphenolic flavonoid, is found in a diversity of plants, especially berry fruits and is a popular nutritional supplement [11]. It has been reported that Res has various biological functions, such as anti-oxidation, anti-inflammation, and anti-carcinogenesis [12-14]. Additionally, Res has been suggested to be protective against cardiac dysfunction induced by sepsis [15]. However, the exact mechanism remains unclear.

The Janus-activated kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway is involved in many physiological processes, including cell survival, inflammation, development, proliferation, and differentiation [16]. It has been suggested that inhibition of the JAK2/STAT3 pathway attenuated multiple organ dysfunction in rats with sepsis [17]. Therefore, JAK2/STAT3 signaling pathway inhibition is beneficial for the treatment of sepsis.
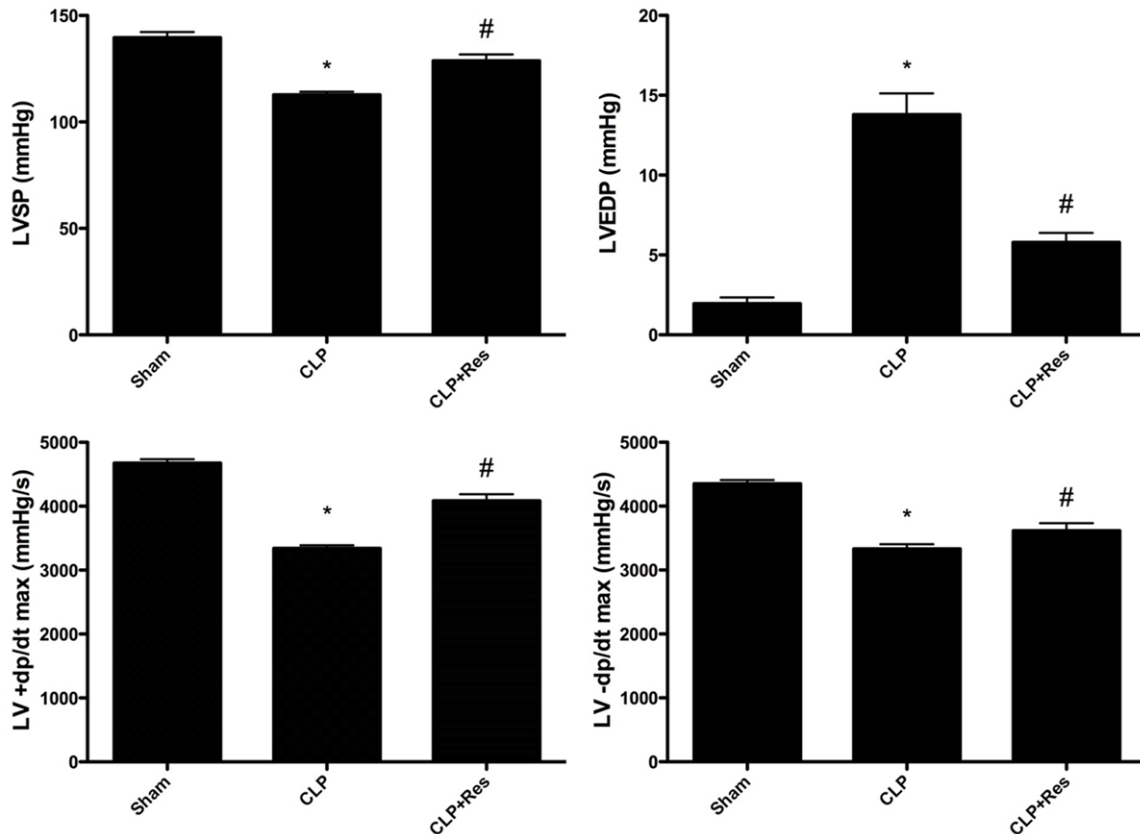
In the present study, cecal ligation and puncture (CLP) was performed in a rat model to investigate the effect of Res on cardiac dysfunction induced by sepsis and the role of JAK2/STAT3signaling pathway in this process.

Materials and methods

Animals and reagents

All experiments were performed on healthy adult male Sprague-Dawley rats that weighed be-

Figure 1. Effect of resveratrol on cardiac function. The results are expressed as the mean ± S.E.M. (n=8 for each group). *P<0.05 in comparison to the sham group, #P<0.05 in comparison to the CLP group. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; LV ± dP/dt$_{max}$, the instantaneous first derivation of left ventricle pressure.

tween 220 g and 250 g. The rats were obtained from the animal center of Jilin University. Rats were kept under pathogen-free conditions at about 22°C on a 12 h light-dark cycle with free access to food and water. This study was performed according to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996).

Res was purchased from Sigma-Aldrich (St. Louis, MO, USA). Rat TNF-α and IL-1β ELISA kits were purchased from Thermo Fisher Scientific (MA, USA). Rat monoclonal antibodies against JAK2 and STAT3 were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Terminal deoxynucleotidyl transferase UTP nick-end labeling (TUNEL) kits were purchased from Roche (Mannheim, Germany).

Cecal ligation and puncture (CLP) model

Fasting was performed for 8 h for all rats but water was allowed ad libitum before the experi-

ments. The CLP model was established as previously reported with some modifications [18]. Briefly, rats were anesthetized with intraperitoneal injection of chloral hydrate (350 mg/kg), and they were immobilized on an aseptic operating table. In a sterile operation environment, a 2-3 cm abdominal midline incision was made to expose the cecum, which was ligated below the ileocecal valve and punctured once with an 18-gauge needle. A small amount of stool was squeezed through the puncture site. The bowel was then situated back in the abdomen and the incision was sutured with a sterile 5-0 silk. The rats in sham-operated group underwent a similar operation without cecal ligation and puncture. All animals received fluid resuscitation with 0.9% saline solution (subcutaneously, 40 mL/kg of body weight) immediately after the surgery.

Experimental protocol

180 rats were randomly assigned to three groups: Sham group received the sham operation
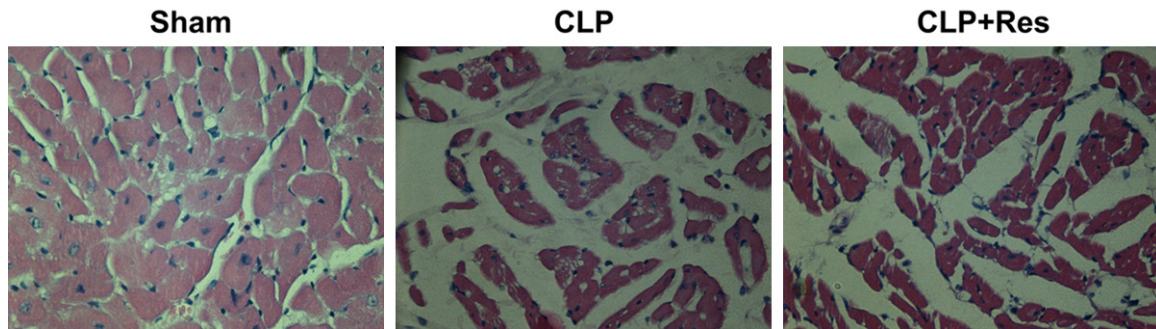
Figure 2. Hematoxylin-eosin staining of myocardial tissue. Representative images of HE staining were shown (magnification: 400×, n=8 for each group). In the sham group, the cardiomyocytes were intact and there was no evidence of necrosis or inflammatory cell infiltration. The cardiac muscle cross striations were clearly visible. In the CLP group, necrosis and inflammatory cell infiltration were evident and the cardiac muscle cross striations were no longer visible. Res administration attenuated the injury induced by CLP.

and no drug treatments; CLP group received the cecal ligation and puncture (CLP) surgery; CLP + Res group received the CLP surgery and Res. Res was administered intraperitoneally at a dose of 60 mg/kg per rat, at 3, 12, and 24 h after surgery.

Cardiac function assessment

A high-fidelity pressure-transducing catheter was inserted via the right carotid artery into the left ventricle to measure the left ventricular pressure (LVP). When the rats returned to stable conditions, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and their first derivative with respect to time ($\pm$ dp/dt$_{max}$) were continuously measured.

Evaluation of morphological changes of myocardial tissue

Rats were sacrificed at 48 h after surgery, and left ventricular myocardial tissues were collected. Tissue sections of the myocardium were stained with hematoxylin-eosin (H&E) staining, and morphological changes were evaluated using light microscopy at a magnification of 400×.

TUNEL staining

Myocardial apoptosis was analyzed using a terminal deoxynucleotidyl transferase UTP nick end labeling (TUNEL) assay. The paraffin-embedded tissue was cut into sections 4-5 µm thick. Then, 50 µL of TUNEL reaction mixture was added to each sample, and the slides were incubated in humidified atmosphere for 60 min

at 37°C in the dark and then rinsed with PBS (pH 7.4) three times, for 5 min each time. To detect the nuclei, the slides were incubated with DAPI for 5 min at room temperature in the dark, rinsed with PBS three times, for 5 min each time, and observed using fluorescence microscopy. The apoptotic index was calculated as the ratio of the number of TUNEL-positive neurons to the total number of nuclei.

Detection of inflammatory cytokines

Inflammatory cytokines in the serum and myocardial tissue were measured 48 h after surgery by using commercially available TNF-α and IL-1β ELISA kits, according to the manufacturer's instructions. The data was analyze using a microplate reader (Multiskan Spectrum, Thermo Scientific, USA).

Western blot

Left ventricular myocardial tissues were collected and lysed with lyse buffer. After sonication, the lysates were centrifuged, and the proteins were separated using SDS-PAGE and then transferred to Immobilon NC membranes (Millipore, Boston, MA, USA). After being blocked with 5% skim milk in Tris-buffered saline at room temperature for 2 h, the membrane was incubated with primary antibodies against JAK2 (1:200), STAT3 (1:200), and β-actin (1:1000) overnight at 4°C, were washed three times with TBST, and then incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at 37°C. The blots were imaged using a Bio-Rad imaging system and quantified using the Qu-
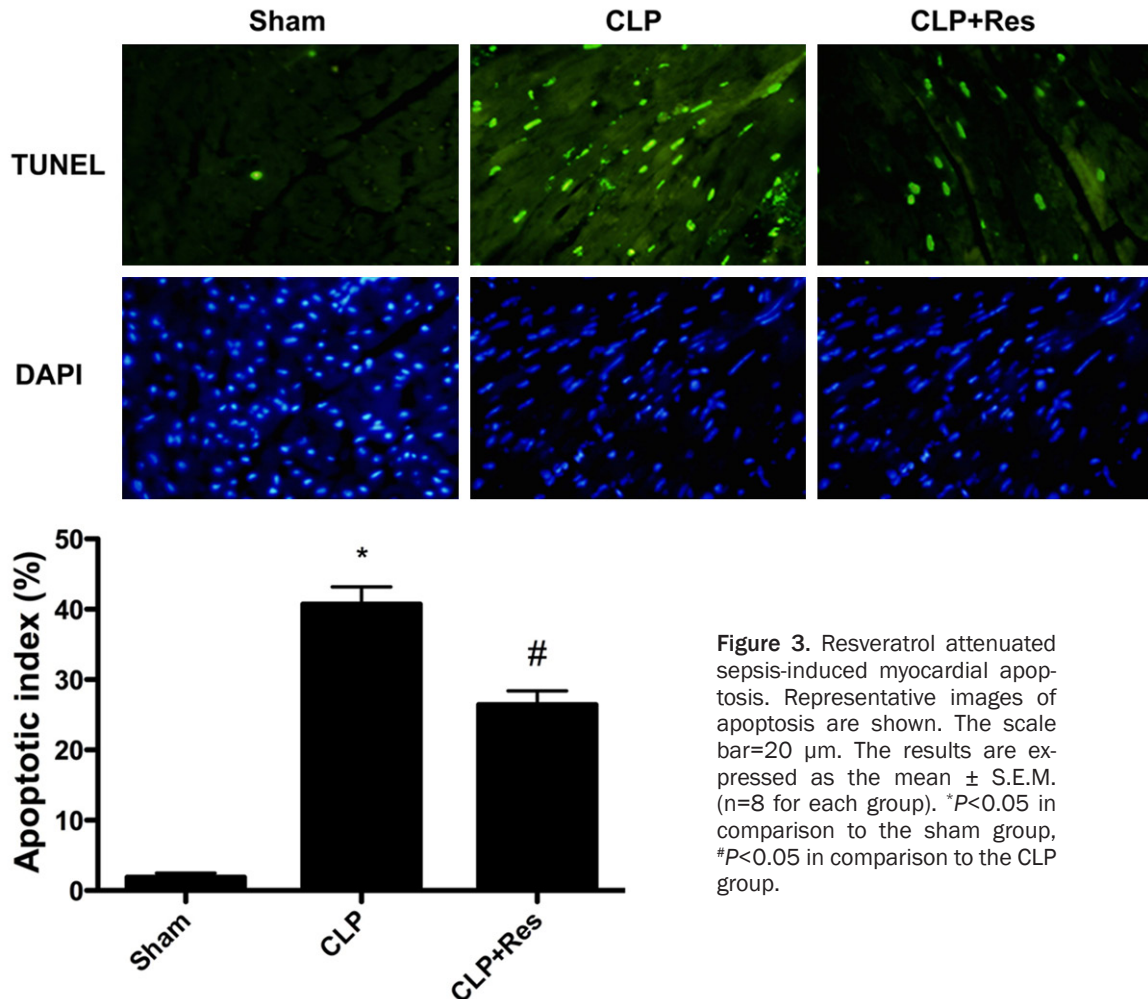
Figure 3. Resveratrol attenuated sepsis-induced myocardial apoptosis. Representative images of apoptosis are shown. The scale bar=20 μm. The results are expressed as the mean ± S.E.M. (n=8 for each group). $^*P<0.05$ in comparison to the sham group, $^#P<0.05$ in comparison to the CLP group.

antity One software package. The value for the sham group was defined as 100%.

Statistical analysis

Data are presented as the mean ± S.E.M. SPSS 18.0 was used to analyze data in this study. Survival rates were calculated using Fisher's exact test. Comparisons among multiple groups were assessed by one-way analysis of variance. The LSD t-test was used to make intergroup comparisons. $P<0.05$ was considered statistically significant.

Results

Resveratrol attenuated sepsis-induced cardiac dysfunction

Sepsis resulted in a severe impairment in cardiac function. As shown in **Figure 1**, the left ventricular systolic pressure and ± dp/dt max were significantly decreased, and the left ventricular end-diastolic pressure was elevated in septic rats in the CLP group compared with the sham group ($P<0.05$). Treatment with Res significantly reversed those detrimental changes ($P<0.05$ versus the CLP group).

Resveratrol attenuated myocardial morphological changes in septic rats

As shown in **Figure 2**, the myocardial tissues were stained with hematoxylin and eosin to evaluate the damage to the myocardium. In the sham group, the cardiomyocytes were intact and there was no evidence of necrosis or inflammatory cell infiltration. The cardiac muscle cross striations were clearly visible. In the CLP group, necrosis and inflammatory cell infiltration were evident and the cardiac muscle cross striations were no longer visible. Res
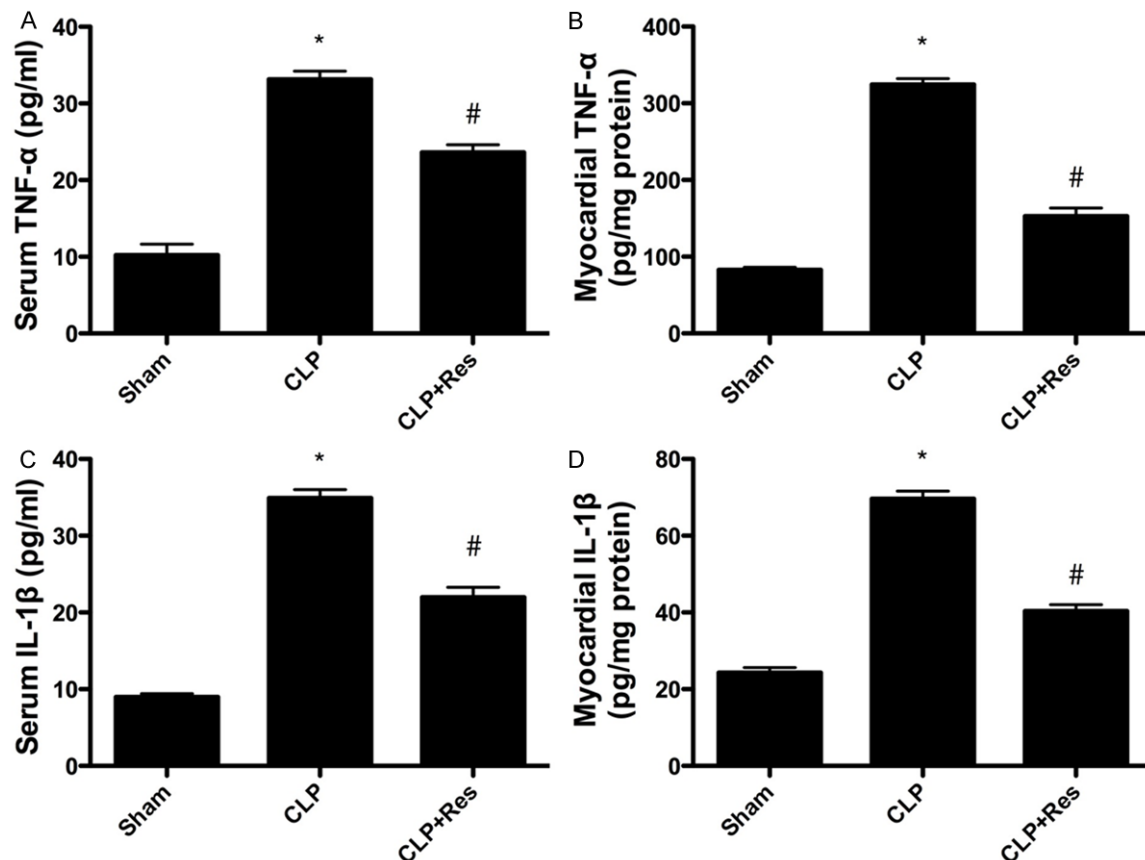
Figure 4. Resveratrol decreased the levels of inflammatory cytokines in the serum and myocardial tissues. A. Serum TNF-α level. B. Myocardial TNF-α level. C. Serum IL-1β level. D. Myocardial IL-1β level. The values are expressed as the mean ± S.E.M. (n=8 for each group). $^*P<0.05$ in comparison to the sham group, $^#P<0.05$ in comparison to the CLP group.

administration attenuated the injury induced by CLP.

Resveratrol alleviated myocardial apoptosis in septic rats

As shown in **Figure 3**, a significant number of TUNEL-positive cells were detected in myocardial tissue in the CLP group compared with the sham group (*P*<0.05). Res significantly reduced the TUNEL-positive staining cells in the CLP + Res group, indicating a significant anti-apoptotic effect of Res (*P*<0.05 versus the CLP group).

*Resveratrol reduced inflammation in septic rats*

As shown in the **Figure 4**, the levels of the inflammatory cytokines TNF-α and IL-1β in the serum and myocardial tissue were markedly increased in septic rats. Those levels were dramatically decreased in the CLP + Res group (*P*<0.05).

Effect of resveratrol on the expression of JAK2 and STAT3

In the **Figure 5**, the Western blot results revealed that the levels of JAK2 and STAT3 were significantly increased in the CLP group (*P*< 0.05). However, Res administration markedly decreased the levels of JAK2 and STAT3 compared with the CLP group (*P*<0.05).

Discussion

Sepsis, the systemic inflammation response syndrome to inflammation, leads to multiple organ failure. Sepsis triggers the activation of various cells, leading to the release of a number of inflammatory mediators, such as cytokines, chemokines, and reactive oxygen species. The excessive production of inflammatory cytokines is out of control, and is associated with numerous signaling pathways, including MAPK pathway, NF-κB pathway, and particularly, JAK/STAT pathway [19-21]. JAK2 and STAT3
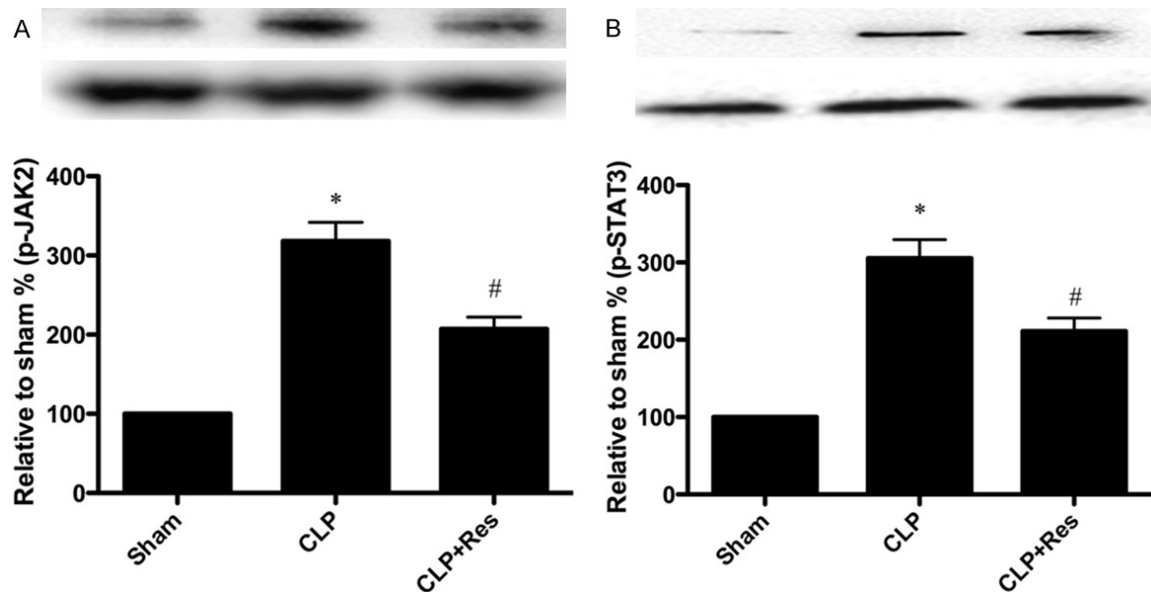
Figure 5. Effect of resveratrol on the expression of JAK2 and STAT3. A. JAK2 expression. B. STAT3 expression. The values are expressed as the mean ± S.E.M. (n=8 for each group). *$P<0.05$ in comparison to the sham group, #$P<0.05$ in comparison to the CLP group.

trigger the release of cytokines, including TNF-α and IL-1β, and are strongly associated with sepsis [18]. In the present study, the results suggest that Res protects the heart from sepsis-induced cardiac dysfunction via JAK2/STAT3 signaling inhibition.

Myocardial dysfunction is considered as an important manifestation of this syndrome [22]. Sepsis patients with myocardial dysfunction are at a 50-70% greater risk of death than patients without cardiovascular complications [23], indicating that myocardial injury is an urgent problem. The underlying mechanisms are multifactorial. Sepsis leads to a reduced cardiac performance [24], resulting in circulatory abnormalities. During sepsis, it is suggested that an accumulation of lipids and glycogen within the cardiomyocytes [25]. The implication is that the dysfunction of the cell that it cannot work normally. The inflammation during sepsis is of great importance, including TNF-α. It has been suggested that the application of murine monoclonal anti-TNF-α antibodies induced a transient improvement in ventricular function in patients with sepsis [26]. As a consequence, the detrimental factors lead to the contractile dysfunction of the heart, contributing myocardial depression during sepsis.

Res has been shown to protect against sepsis. Wang et al. suggest that Res attenuates micro-vascular inflammation in sepsis [27]. Res attenuates lipopolysaccharide-induced acute kidney injury [28]. Res is reported to alleviate endotoxin-induced myocardial toxicity via the Nrf2 transcription factor [29]. Res reduces acute lung injury in an LPS-induced sepsis mouse model via activation of Sirt1 [30]. In our study, Res improves the survival rate and cardiac function in septic rats, and Res attenuates apoptosis and inflammation during sepsis.

JAK2/STAT3 signaling pathway plays an important role in inflammatory responses. The activation of JAK2/STAT3 signaling can trigger the release of TNF-α and IL-1β, leading to the overwhelming inflammation during sepsis. In the present study, we found that the expression of JAK2 and STAT3 were increased significantly after sepsis. After treatment with Res, the levels of the two proteins decreased, suggesting that resveratrol protects against sepsis via inhibition of JAK2/STAT3 signaling.

In conclusion, our results suggest that Res improves survival rate and cardiac dysfunction during sepsis, and suppresses apoptosis, TNF-α and IL-1β production. The JAK2/STAT3 signaling inhibition is involved in the protective effects of Res. Our work provides evidence for the clinical use of Res in the treatment of sepsis-induced myocardial dysfunction.

Disclosure of conflict of interest

None.

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