Inflammation, Vol. 42, No. 6, December 2019 (© 2019)
DOI: 10.1007/s10753-019-01081-3

ORIGINAL ARTICLE

SS31 Ameliorates Sepsis-Induced Heart Injury by Inhibiting Oxidative Stress and Inflammation

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Abstract—Sepsis-induced myocardial dysfunction (SIMD), lack of effective treatment, accounts for high mortality of sepsis. Mitochondrion-targeted antioxidant peptide SS31 has been revealed to be responsible for certain cardiovascular disease by ameliorating oxidative stress injury. But whether it protects a septic heart remains little known. This study sought to prove that SS31 was capable of improving sepsis-induced myocardial dysfunction dramatically. C57BL/6 mice were intraperitoneally administered lipopolysaccharide (LPS), exposed to systemic inflammation. Thirty-five C57BL/6 mice were randomly divided into four groups: sham group, LPS group (5 mg/kg), SS31 group (5 mg/kg), and SS31 + LPS group (treatment group). Heart tissues were harvested for pathological examination at the indicated time points. H9C2 cell were treated with LPS with or without the presence of SS31 (10 μM) at 37 °C to assess the effect on cardiomyocytes at the indicated time points. SS31 restored myocardial morphological damage and suppressed inflammatory response as evidenced by significantly decreasing the mRNA levels of IL-6, IL-1β, and TNF-α *in vitro* and *in vivo*. In addition, myocardial energy deficiency secondary to sepsis was remarkedly ameliorated by SS31. Furthermore, we found that SS-31 normalized the activity of malondialdehyde, glutathione peroxidase, and superoxide dismutase *in vitro* and *in vivo*, and maintained mitochondrial membrane potential (MMP) as well. And western blot was applied to measure the expressions of p-p38MAPK, p-JNK1/2, p-ERK, p62, and NF-κB p65; the results illuminated that the cardioprotective effect of SS31 was partly linked to NF-κB. In conclusion, SS31 therapy effectively protected the heart against LPS-induced cardiac damage.

KEY WORDS: SS31; SIMD; mitochondria; inflammation; oxidative stress.

INTRODUCTION

Sepsis is a lethal syndrome caused by a series of inappropriate immune responses [1, 2], which is responsible for million deaths annually [3, 4]. Sepsis may develop into a kind of systemic inflammatory response syndrome (SIRS) without properly and promptly control, eventually leading to multiple organ dysfunction (MOD). Sepsis-induced cardiomyopathy (SIC) is one of common complications of sepsis, characterized by high morbidity and mortality [5]. Patients with cardiac dysfunction are

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10753-019-01081-3) contains supplementary material, which is available to authorized users.

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undergoing compromising myocardial contractility, decreased left ventricular ejection fraction, and reversible biventricular dilation [2, 6]. Although sepsis and SIC have long been recognized, there is still no efficient therapy, in addition to antibiotics and restoration of blood pressure and organic perfusion [7–9].

It has been reported that sepsis-induced cardiac injury was a consequence of uncontrolled inflammation [10], mitochondrial dysfunction [11], oxidant/antioxidant imbalance [12], excessive apoptosis [13], and autonomic nervous system malfunction [2, 6, 14]. An increasing body of evidence has indicated that oxidative stress, inflammatory damage, and mitochondrial dysfunction play a pivotal role in the pathogenesis of sepsis-induced cardiac dysfunction. At the early onset of SIC, cardiac mitochondria display abnormalities such as swelling, loss of cristae, cleared matrix, internal vesicles, and rupture of the inner and outer membranes, and alterations that persisted up to 24 h [15]. Ultrastructural abnormalities of myocardial mitochondria are deleterious mechanisms in production of the bulk of energy needed by the cell for normal function. Notably, increased ROS production coming from mitochondria, along with Ca^{2+} overload, triggers the opening of the mitochondrial permeability transition pore (mPTP). The mPTP opening, as described before, induces externalization of mtDNA fragments and activates the inflammation pathway subsequently [16]. Although mitochondrial impairment has been well-described in SIC, mitochondrion-targeted management is still absent from current clinical practice because of its failure to target mitochondria.

SS31 peptide (H-D-Arg-Dmt-Lys-Phe-NH2), a novel mitochondrial-targeting antioxidant peptide, has been unveiled to assume critical roles in cardiac pathophysiology and cardiovascular disease. The protective effects of SS31 in myocardial infarction [17], atherosclerosis [18], and hypertrophic cardiomyopathy [19] have already been studied. Cho et al. have put forward that SS31 exhibited a significant cardioprotective effect, ameliorating oxidative stress injury [17]. And other researches revealed that SS31 could also attenuate inflammation, inhibit cell apoptosis, and suppress the Ca^{2+}-induced mitochondrial permeability transition (MPT) [20, 21]. Nevertheless, whether SS31 has a protective effect on SIMD remains to be elucidated. A better understanding on the effect of SS31 in SIC may guide future treatments in this field. In the present study, we construct a model of LPS-induced sepsis in mice and investigate the protective role of SS31 against sepsis-induced cardiac dysfunction.

MATERIALS AND METHODS

Animals

Eight- to 10-week-old male C57BL/6 mice (17–25 g) were obtained from the Institute of Laboratory Animal Science, Hubei University of Medicine (Shiyan, China). Before the experiments, all the mice were adapted for more than 1 week. And all the procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication, revised 2011) and were approved by the Ethical Committee for Animal Experimentation of Xiangyang No.1 People's Hospital.

Experimental Grouping and Treatment

Thirty-five C57BL/6 mice were randomly divided into four groups: sham group, LPS group (5 mg/kg *Escherichia coli* lipopolysaccharide, serotype 0111: B4, Sigma; dissolved in 0.9% NaCl; intraperitoneal injection, i.p.), SS31 group (5 mg/kg SS31; China Peptides Co., Ltd., Shanghai, China; dissolved in dissolved in ddH2O, i.p.), and SS31 + LPS group (5 mg/kg SS31, 5 mg/kg LPS, i.p.). In SS31 + LPS group, SS31 was intraperitoneally administered 30 min following LPS. The dose of SS-31 was used according to a previous study [22]. Subsequently, all hearts were excised after the mice were euthanized at different time periods. Half of the hearts in each group were prepared for tissue homogenate, and the other half were utilized for histopathological evaluation.

Cell Culture and Reagents

H9C2 cardiomyoblasts were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, NY, USA), supplemented with 10% fetal calf serum (Siji Qing, China), penicillin (100 U/ml), and streptomycin (100 mg/ml) (Gibco, 15140) at 37 °C in a humidified 5% CO_2 atmosphere.

Histological Staining

The myocardium was fixed in 4% paraformaldehyde and sectioned at a thickness of 4–5 μm. Morphological changes in myocardial tissues were observed by hematoxylin-eosin (H&E) staining under a light microscope. Three hearts were analyzed per group. A blinded examiner performed the analysis of the histology slides.

TUNEL Staining

Visualization of apoptotic cardiomyocytes was performed on left ventricular tissue cross sections (16 μm thick) of LPS group, SS31 group, treatment group, and sham group ($n = 8$ per group) by TdT-mediated dUTP-biotin nick-end labeling (TUNEL) method, using the TUNEL Apoptosis Detection Kit (Roche) and according to the manufacturer's procedure. Sections were then counterstained with hematoxylin for 5 min for nuclear tissue. Cells with a brown-red nuclear labeling were defined as TUNEL-positive. Positive controls were provided by sections pretreated with DNAse I Buffer (100 U/ml) for 10 min at 15–25 °C to induce DNA strand degradation. In negative control experiments, TdT was omitted from the labeling mixture, and no staining was detected. TUNEL index in each region = (100% × [number of TUNEL-positive nuclei/total number of nuclei]).

Real-time PCR analysis

According to the manufacturer's instructions (Invitrogen), we used TRIzol reagent to purify total RNA from cells or tissues. An oligo dT primer and Transcriptor First Strand cDNA Synthesis Kit (Promega (Beijing) Biotech Co., China) acted synergistically in reversing transcribed 2 μg total RNA into cDNA. Amplification and quantitative RT-PCR analyses were performed in SYBR Green (Promega, USA) on a 7500 cycler (ABI, USA). The relative mRNA expression of target genes was normalized to GAPDH. Specific primer sequences used in the study were listed in Table 1. The relative mRNA expression level was determined by calculating the values of the Δcycle threshold (ΔCt) by normalizing the average Ct value to that of the endogenous control (GAPDH or β-action), and then calculating $2^{-\Delta\Delta Ct}$ values.

ROS content

ROS assay kit (Beyotime Institute of Biotechnology, China) was used to examine the intracellular level of ROS. Briefly, H9C2 cardiomyocytes were seeded on a twelve-well culture plate and then were treated with or without LPS (10 mg/ml; Biosharp Life Sciences, China; dissolved in ddH_2O) or SS31 (30 μM; China Peptides Co., Ltd., Shanghai, China; dissolved in ddH_2O) for 6, 12, and 24 h. Then, cells were added to 10 μM DCFH-DA to completely cover the cells over 20 min. We observed cells by laser confocal microscopy following washing cells three times with serum-free cell culture medium.

Antioxidant Enzyme Assays

The heart tissues or H9c2 cells were lysed on ice in RIPA lysis buffer (Beyotime Institute of Biotechnology, China) supplemented with protease inhibitor pellets (Roche). The supernatants of lysates were obtained by centrifugation at 12000*g* for 15 min at 4 °C, and the concentrations were measured with a BCA-kit (23227, Thermo Fisher Scientific, Waltham, MA). Then, the supernatant was collected on ice and stored at − 80 °C in a freezer. Superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) activities were estimated by kits (Nanjing Jiancheng Bioengineering Institute, China). After adding the corresponding reagents as the order of the instructions, the OD value of each well was recorded through a microplate reader and the corresponding value was calculated.

Mitochondrial Function

5,5′,6,6′-Tetrachloro-1,1′,3,3′ tetraethylbenzimidazolylcarbocyanine iodide (JC-1) mitochondrial membrane potential detection kit (Beyotime Institute of Biotechnology, China) was used to determine the mitochondrial membrane potential (MMP) level. Six-well culture plates of H9C2 cardiomyocytes were co-reacted with JC-1 (2 μg/ml) for 5 min in 37 °C incubator. After washing twice with PBS, the cardiomyocytes were observed under a laser confocal microscope. The ratio of red fluorescence to green fluorescence represented MMP. Assessment of relative ATP contents was performed by the ATP assay kit (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's instructions.

Western Blot Analysis

Samples were loaded onto an SDS-PAGE gel (Beyotime Institute of Biotechnology, China) and transferred to PVDF membranes electrophoretically (Millipore, Billerica, MA, USA). Five percent non-fat milk was used to block the membranes in Tris-buffered saline Tween 20 (TBST) for 1 h. The membranes were incubated with primary antibodies overnight, including phospho-p38MAPK (1:1000, Absin), phospho-JNK 1/2 (1:1000, Absin), phospho-ERK (1:1000, Absin), p62 (1:1000, Absin), NF-κB p65 (1:1000, Absin), TNF-α (1:1000, Absin), and GAPDH (1:1000, Absin) antibodies. Subsequently, secondary antibodies reacted with the bolts at room temperature for 2 h before detecting with chemiluminescence ECL kit (Beyotime Institute of Biotechnology, China).

Table 1. Primers Used for Real-time RT-PCR

Gene	Species	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
IL-6	Mouse	CCGGAGAGGAGACTTCACAG	TCCACGATTTCCCAGAGAAC
IL-1β	Mouse	TCACAGCAGCACATCAACAA	TGTCCTCATCCTGGAAGGTC
TNF-α	Mouse	ACGGCATGGATCTCAAAGAC	GTGGGTGAGGAGCACGTAGT
iNOS	Mouse	AGGGAATCTTGGAGCGAGTT	GCAGCCTCTTGTCTTTGACC
Atg3	Mouse	TGATGGGGGATGGGTAGATA	CAGTGCTGAGCAATCTTGGA
P62	Mouse	CCCTCTAGGCATTGAGGTTG	GCTGCTTGGCTGAGTGTTAC
Beclin-1	Mouse	ATGTGGAAAAGAACCGCAAG	ACTCCAGCTGCTGCCTTTTA
β-Actin	Mouse	GCAGCTCCTTCGTTGCCGGT	ACATGCCGGAGCCGTTGTCG
IL-6	Rat	GTTGCCTTCTTGGGACTGATG	ATACTGGTCTGTTGTGGGTGGT
IL-1β	Rat	CCGTGGACCTTCCAGGATGA	GGGAACGTCACACACCAGCA
TNF-α	Rat	AGCATGATCCGAGTGTGGAA	TAGACAGAAGAGCGTGGTGGC
GAPDH	Rat	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT

Statistical Analyses

All data coming from our study were presented as the means ± SEM, and the GraphPad Prism software version 8.0 was used to analyze the statistics. The one-way ANOVA method was applied to analyze statistical significance. p values less than 0.05 was considered statistically significant.

RESULTS

SS31 Resisted Myocardial Morphological Damage and Apoptosis in Sepsis Stimulated by LPS

Intraperitoneal injection of LPS *in vivo* mediates cardiomyocyte damage. Consistent with previous research, cardiomyocytes in LPS group displayed an apparent feature of myocardial damage after the challenge of LPS for 12 h, as evidenced by the extent of apoptosis and inflammatory cell infiltration (Fig. 1). There were no significant differences between the sham group and the SS31 group. However, cardiomyocytes in the LPS group displayed an apparent feature of myocardial damage after the challenge of LPS for 12 h, as evidenced by the extent of necrosis and inflammatory cell infiltration and less visible myocardial cross-striations. With the administration of SS31, myocardial morphological changes improved significantly compared with that of the LPS group (Fig. 1). We further found that septic mice showed a robust rise of cardiomyocyte apoptosis according to TUNEL staining. In sham and SS31 groups, only particularly rare TUNEL-positive cells were identified in the media. However, in the treatment group, there were significantly fewer numbers of TUNEL-positive cells, compared with that in the LPS group (Fig. 1b). Consequently, apoptotic index was increased and attained approximately 60% ($p < 0.05$) in the LPS group (Fig. 1c). Apoptotic index decreased extensively by SS31 treatment ($p < 0.05$). Thus, SS31 alleviated septic myocardial damage.

SS31 Exhibited Declining Expression of Inflammatory Cytokine After LPS Stimuli

Then, we investigated whether administration of SS31 could influence inflammatory process during sepsis. As presented in Fig. 2, the mRNA levels of inflammatory cytokines (IL-1β, IL-6, and TNF-α) increased significantly following the administration of LPS *in vivo* and *in vitro*. Moreover, the upregulation of the inflammatory cytokines in H9C2 induced by LPS was restored by SS31 ($p < 0.05$; Fig. 2b). These results revealed that the treatment of SS31 decreased the expression of pro-inflammatory cytokines compared with that of the LPS group ($p < 0.05$; Fig. 2a). However, it seemed that SS31 had no significant time-dependent effect during sepsis (Fig. 2a, b).

SS31 Ameliorated Oxidative Stress Induced by LPS *In Vitro* and *In Vivo*

We also tested the myocardial reactive oxygen species (ROS) in this study. As shown in Fig. 3a, the ROS content in the LPS group increased significantly compared with that in the con group after 12 h. Although LPS exposure induced ROS overproduction, the treatment with SS31 inhibited ROS production significantly compared with that of the LPS group (Fig. 3a). However, SS31 was less efficient after 24 h. In line with the results from the levels of ROS, the compromising activities of SOD and GSH-Px induced by LPS were reversed following the treatment of SS31 as well *in vivo* and *in vitro* (*vs.* LPS group, $p < 0.05$; Fig. 3b).

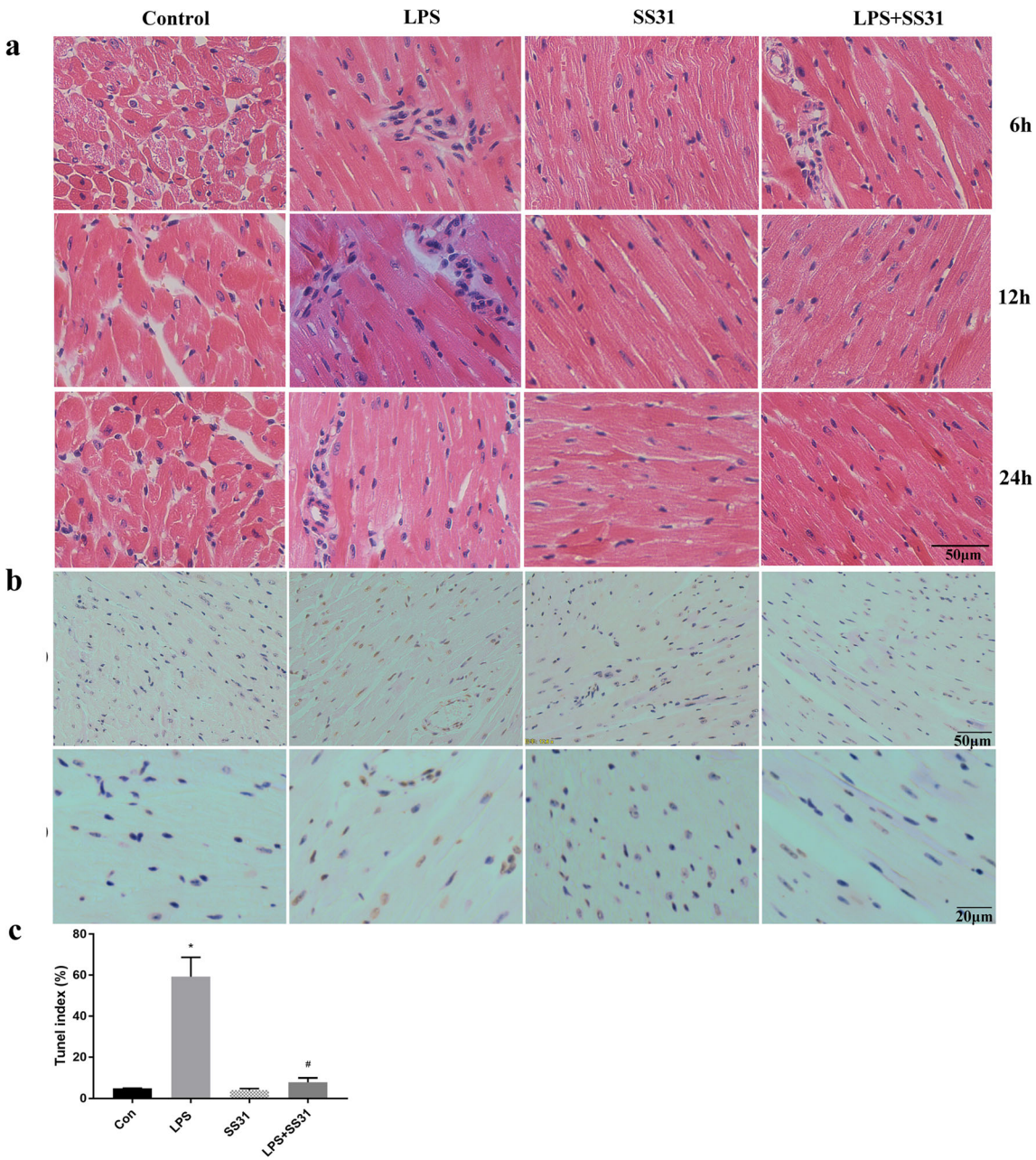

Fig. 1. SS31 resisted myocardial morphological damage and apoptosis in sepsis stimulated by LPS. Mice were intraperitoneally administered 5 mg/kg SS31, or vehicle (ddH_2O) 0.5 h following LPS. Heart tissues were harvested and then sectioned for H&E and TUNEL staining. Representative images were chosen from different groups. **a** The microscopic findings (× 200) of H&E staining identified that there was inflammatory infiltration and cardiomyocyte disarrangement. **b** TUNEL assay and **c** TUNEL index from different groups. The microscopic findings (× 200 and × 400) of TUNEL staining were shown. Data are expressed as mean number of apoptotic cells per field. Three hearts were analyzed per group. $^{*}p < 0.05$ *versus* the con group; $^{\#}p < 0.05$ *versus* the LPS group.

SS31 Increased Cardiac ATP Levels and Preserved the Stability of MMP After LPS

As shown in Fig. 4a, mitochondrial membrane potential depolarization was induced by LPS exposure, which led to cell apoptosis and mitochondrial damage. Also, MMP in the LPS + SS31 group showed no apparently differentiation compared with that in the control group. Meanwhile, the ATP level was visibly downregulated after

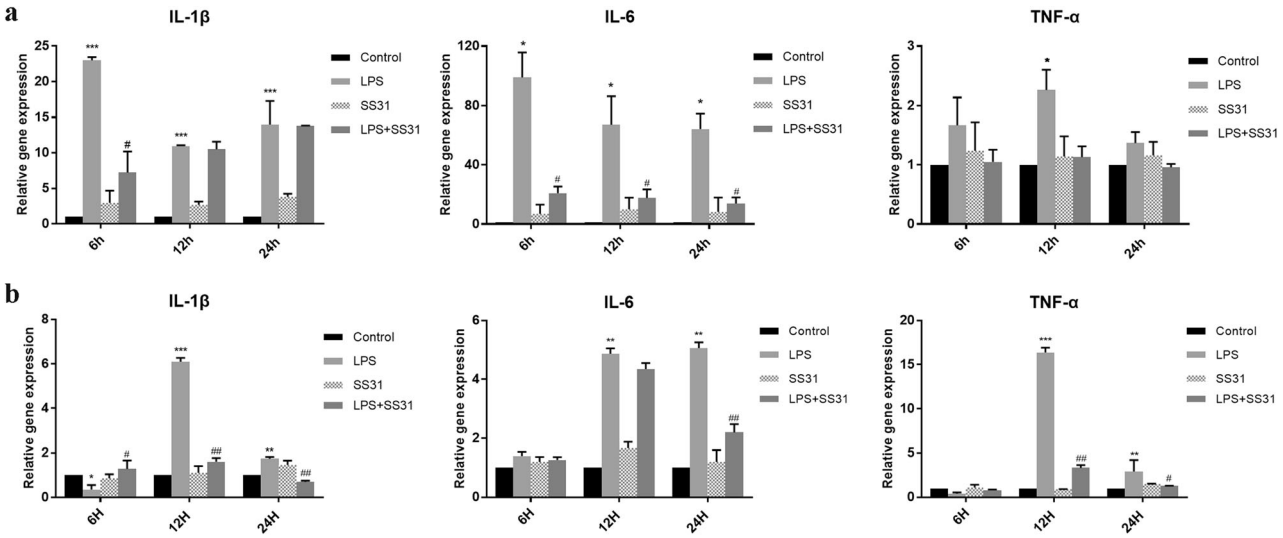

Fig. 2. SS31 attenuated LPS-mediated cardiac inflammatory responses. The mRNA expression of pro-inflammatory cytokines was quantified by RT-PCR at 6 h, 12 h, and 24 h after LPS. **a** C57BL/6 mice were injected with LPS, SS31, or vehicle. **b** H9C2 cells were stimulated with or without LPS (gray bars) or LPS + SS31(dark gray bars) for 6 h, 12 h, and 24 h. Cellular experiments were repeated 3 times independently. Data are presented as the mean ± SEM ($n = 8$ per group). $^{*}p < 0.05$ *versus* the con group; $^{\#}p < 0.05$ *versus* the LPS group.

LPS exposure within 6 h and upregulated by treatment of SS31 (Fig. 4b). However, there was no clear time-dependent effect with SS31.

NF-κB Might Inhibit Cardioprotective Role of SS31 in Part

It was well-known that multiple signaling pathways were involved in the process of sepsis, such as the NF-κB and MAPK signaling pathways [23]. Immunoblots were as well performed to estimate p-P38MAPK, JNK1/2, p-ERK, p62, and endonuclear (Nuc-P65) p65. As shown in Fig. 5, the phosphorylation and nuclear translocation of p65 were increased by LPS exposure, which would also be depressed by administration of SS31 (Fig. 5). However, no observed difference was detected between LPS and LPS + SS31 groups in p62, JNK1/2, and p-p38MAPK levels.

DISCUSSION

Sepsis is the world's leading killer; five of the top 10 WHO causes of death fulfill the definition of sepsis. SIC has become the major health problems worldwide [24]. SIC is known to associate with myocardial mitochondrial structure damage and loss of mitochondrial density [25]. Currently, little is known regarding reducing myocardial mitochondrial damage to improve sepsis outcomes. The major findings from our study revealed SS31 administration significantly reduced inflammation and apoptosis and preserved MMP, to reduce mitochondrial damage, and thus improved cardiac function in LPS-induced mice. Moreover, SS31 administration markedly inhibited NF-κB signal pathway activated by LPS, further downregulated excessive inflammatory process. These data indicate the potential application of mitochondrion-specific targeted antioxidant SS31 in protecting cardiomyocytes from LPS-induced inflammatory process, mitochondrial functional deficiency, and destruction of redox balance.

Mitochondrial function plays a critical role in the pathogenesis of sepsis, to be repressed by myocardiodepressant factor in both septic animals and patients. Myocardial depressant factors, such as IL-1β, TNF-α, and ROS, are committed to aggravating mitochondrial damage, and studies have demonstrated that ameliorating oxidative stress and inflammation attenuates mitochondrial dysfunction [24]. Excess inflammatory mediators not only lead to mitochondrial dysfunction, but also stimulate the production of peroxides, causing adjacent tissue injury, cardiac dysfunction, multiple organ failure, and death [14]. To our knowledge, anti-infection treatment neither significantly improve the survival rate of patients with sepsis. It has been reported that blocking IL-1 specifically failed to reduce morbidity and mortality in sepsis [14]. In addition, anti-TNF-α treatment succeed in improving survival in septic animal models, but failed to attenuate mortality

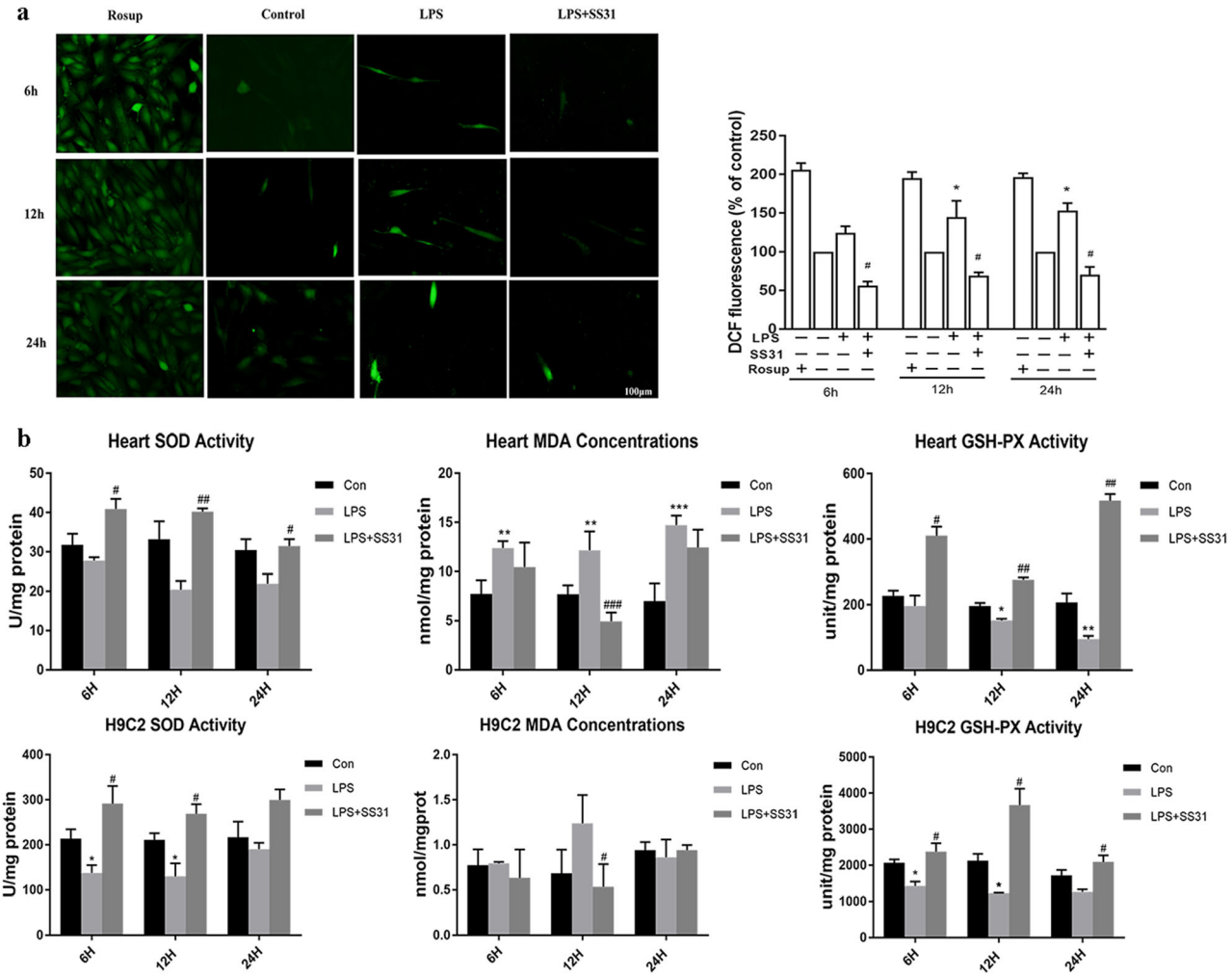

Fig. 3. SS31 blunted the effects of LPS on oxidative stress *in vitro* and *in vivo*. **a** H9C2 cells were treated at 37 °C in the absence or presence of LPS (10 mg/ml) or SS31 (30 μM) for 6 h, 12 h, and 24 h, then incubated with DCFH-DA and observed by a laser scanning confocal microscope. Representative images (× 200) were chosen from different groups. **b** Relative levels of SOD, MDA, and GSH-Px in each group. The graph shows mean ± SE, $n = 8$/group; cellular experiments were repeated 3 times independently. $^{*}p < 0.05$ *versus* the con group; $^{\#}p < 0.05$ *versus* the LPS group.

in septic patients [3]. Drosatos et al. also had highlighted that administration of anti-inflammatory therapy in clinical trials failed to reduce the overall mortality of patients with sepsis, although anti-inflammatory therapies were so important [3, 26]. Our study demonstrated that SS31 significantly inhibited inflammatory factors according to the decreased number of IL-1β, TNF-α, and IL-6. Among the many kinases, NF-κB signaling pathway is reported to account for a series of cardiac inflammatory responses during sepsis [27]. In fact, we discovered that SS31 partially inhibited NF-κB. SS31 might downregulate the RNA expression of pro-inflammation factors caused by LPS via regulating the NF-κB signaling pathway.

SS31 is an innovative cell-permeable mitochondrion-targeted antioxidant peptide. SS-31 is known to concentrate in the inner mitochondrial membrane more than 1000-fold compared with the cytosolic concentration. A previous study demonstrated that SS-31 might scavenge ROS directly at the site of their production, and bind to cardiolipin via electrostatic and hydrophobic interactions and thereby protected the mitochondrial function, increasing the efficiency of mitochondrial electron transport [28] and attenuating mtROS production [29]. Increased efficiency of ATP generation and reduction in ROS are thereby coupled. Our study demonstrated that SS31 administration significantly upregulated the production of ATP *in vivo*

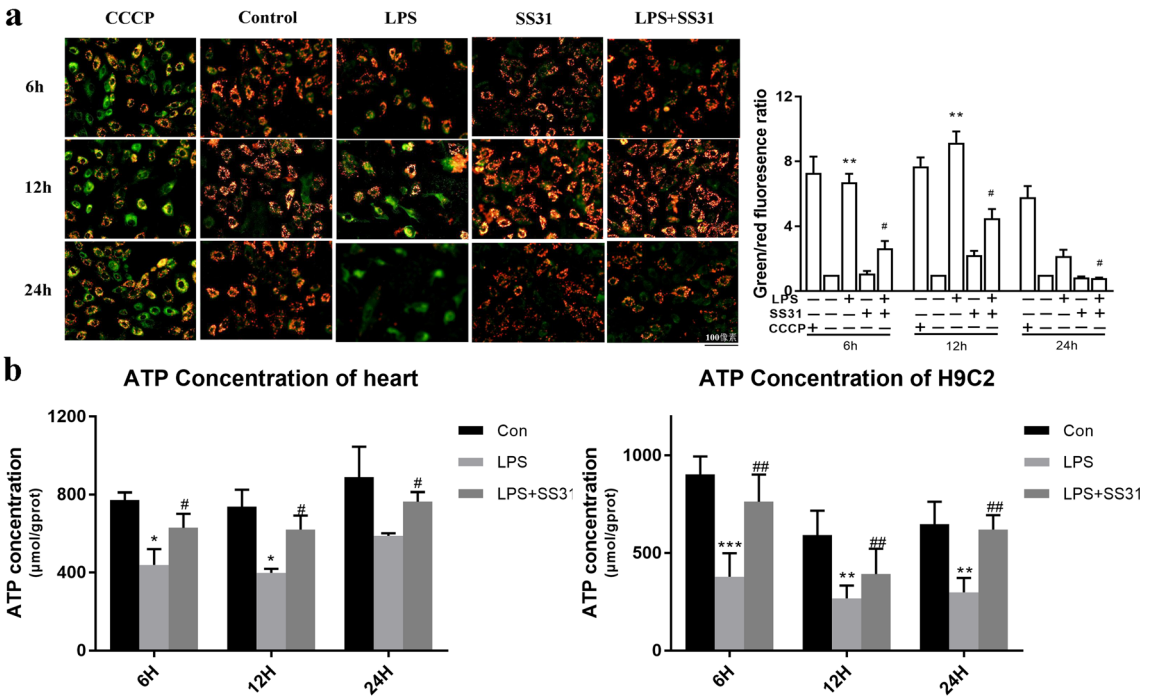

Fig. 4. SS31 reversed mitochondrial dysfunction caused by LPS. **a** Mitochondrial membrane potential (MMP) images are shown. Representative images (× 200) were chosen from each group at different time points. **b** SS31 improved cardiac energy metabolism *in vivo* and *in vitro*. Cardiac and H9C2 myocardial cell ATP content in the indicated groups ($n = 8$). Data are presented as the mean ± SEM. $^{*}p < 0.05$ *versus* the control group; $^{\#}p < 0.05$ *versus* the LPS group. Cellular experiments were repeated 3 times independently.

and *in vitro* accompanying reducing ROS content of myocardial cell in septic mice.

Furthermore, our results are in accordance with the study of Li et al. [22], which showed that SS31 protected against sepsis-induced organ dysfunctions, and alleviated the survival rate. But, the cardioprotective effect of SS31 has rarely illuminated by Guoming Li. And we found that SS31 was advantageous to the septic cardiomyocytes due to inhibiting the inappropriately opening of mitochondrial permeability transition pore, and maintaining the stability of MMP. Besides, SS31 largely corrected the activities of SOD, GSH-PX, and MDA to balance the oxidative status, supporting the effect of SS31 in sepsis.

Of note, cardiac dysfunction represents a clinical feature of sepsis. Cardiomyocyte apoptosis is one of the major pathogenic mechanisms underlying myocardial injury and cardiac dysfunction caused by sepsis [30]. Previous studies have suggested that lower ejection fraction (EF) and fractional shortening (FS) and higher left ventricle end-diastolic volume (LVESV) during sepsis were closely related to myocardial cell apoptosis, which were essential for increasing mortality [13]. Apoptosis in cardiac myocytes is associated with mitochondrial dysfunction [31]. As expected, we found that sepsis induced cardiomyocyte apoptosis in LPS-induced mice. SS31 abolished these injuries in septic mice. However, cardiac function indexes represented by EF, FS, and LVESV were not investigated in our article, which was our shortcomings.

The cardiomyocytes with defects in autophagy have resulted in imbalance of proteostasis, which was associated with progression of cardiac disease [32]. Myocardial mitochondrial autophagy exerted protective effects on cardiac dysfunction subjected by sepsis [33]. We, however, were unable to prove that SS31 restored cardiac performance by extensively improving autophagy, according to the levels of beclin-1, p62, and Atg3 (supplement figures).

This article has certain limitations that should be acknowledged. We did not measure the indicators of cardiac function, such as LVESV, EDV, and LVEF, as well as vital biomarkers of myocardial injury. Additionally, follow-up studies relative to the survival rates with the treatment of SS31 will be needed to fill in this gap. Further investigation of SS31 needs to be carried out to develop the whole picture of its mechanism of action.

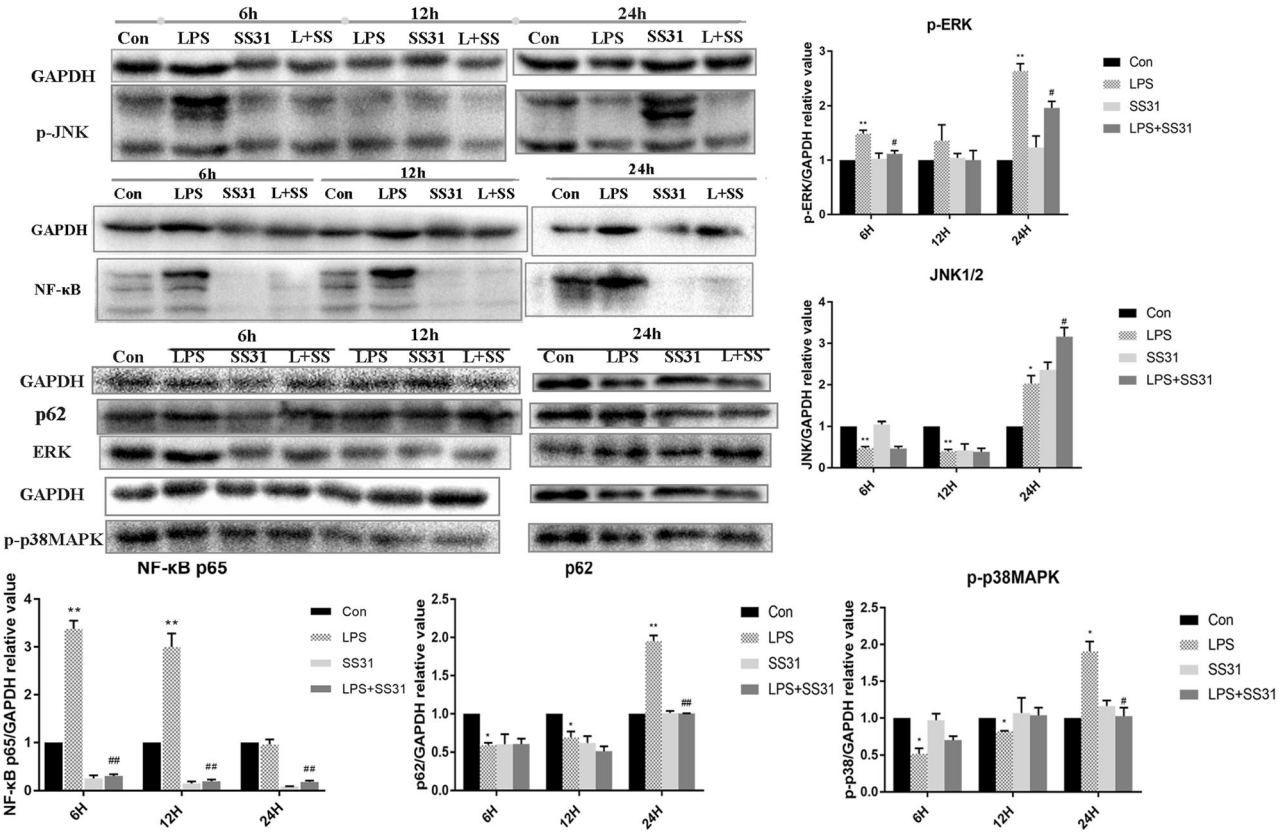

Fig. 5. Effects of SS-31 on signal pathway. The heart tissues were collected for the detection of p-P38MAPK, JNK1/2, p-ERK, p62, and NF-κB p65 at 6 h, 12 h, and 24 h after LPS by western blotting. **a** Representative blots of phosphorylated p38MAPK, phosphorylated JNK1/2, phosphorylated ERK, p62, and NF-κBp65 signaling pathway. **b** Quantification of these proteins showed by fold change ($n = 8$). All of the proteins were normalized to GAPDH before quantification; data are presented as the mean ± SEM, $n = 8$ per group. $^{*}p < 0.05$ *versus* the control group; $^{\#}p < 0.05$ *versus* the LPS group.

Taken together, the salient finding of the present study is that SS31 improves oxidant/antioxidant imbalance, energetic starvation, apoptosis, and mitochondrial dysfunction caused by sepsis. Our study provides basic evidence that SS31 may serve as a mitochondrial protectant for sepsis-induced myocardial dysfunction. Considerably more work will need to be done to the future clinical use of SS31 in sepsis.

AUTHORS' CONTRIBUTIONS

R.J., M.S., Y.L., and X.D.S. conceived and designed this study. Y.L. carried out experiments. W.J.Y., Y.Y., and L.X.X. collected and analyzed data. W.J.Y. performed statistical analysis. Y.L. and W.J.Y. wrote the manuscript, which was critically reviewed and revised by R.J. and M.S. All authors read and approved the final manuscript.

FUNDING INFORMATION

This investigation was supported by the grants from the Natural Science Foundation of Hubei Province of China (2017CFB674, 2018CFB701), Science and Technology Plan Project of Xiangyang (2014-7-16), the Innovative Team Project (2017-2019) from the Institute of Medicine and Nursing at Hubei University of Medicine, and the Initial Project for Ph.D of Hubei University of Medicine (2015QDJZR03).

COMPLIANCE WITH ETHICAL STANDARDS

And all the procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication, revised 2011) and were approved by the Ethical Committee for Animal Experimentation of Xiangyang No.1 People's Hospital.

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

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