Life Sciences 217 (2019) 8–15

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Melatonin protects against sepsis-induced cardiac dysfunction by regulating apoptosis and autophagy via activation of SIRT1 in mice

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ARTICLE INFO

Keywords:
Sepsis
Cardiac dysfunction
Melatonin
Apoptosis
Autophagy
SIRT1

ABSTRACT

Aims: The apoptosis and autophagy play an important role in the pathogenesis of sepsis-induced cardiac dysfunction. Previous studies have demonstrated that melatonin protects against cardiac dysfunction during sepsis. In addition, silent information regulator 1 (SIRT1) is a therapeutic target for sepsis-induced myocardial dysfunction. The aims of this study were to investigate whether SIRT1 was involved in melatonin's cardioprotection during sepsis and the mechanisms.

Materials and methods: In this study, twenty-four male C57BL/6 mice were randomly assigned to four groups: Control group, LPS group, LPS + Melatonin group and LPS + Melatonin + EX527 group. Mice were treated with lipopolysaccharide for 8 h with or without melatonin or EX527. The cardiac function, myocardial injury biomarkers, cardiac histopathology, cardiomyocyte apoptosis, autophagosome as well as the protein expressions of SIRT1, cleaved caspase-3, LC3-II/LC3-I ratio and p62 in the myocardium were assayed.

Key findings: The results demonstrated that melatonin significantly improved cardiac function, decreased creatine kinase (CK) and creatine kinase-MB (CK-MB) levels, attenuated myocardial architecture destruction, inhibited cardiomyocyte apoptosis and increased cardiac autophagy as compared with the LPS group. In addition, melatonin significantly increased SIRT1 protein expression in the myocardium of mice with sepsis, while inhibition of SIRT1 by EX527 abolished melatonin's cardioprotection during sepsis.

Significance: In this study, we found that melatonin protected against sepsis-induced cardiac dysfunction by regulating apoptosis and autophagy via activation of SIRT1 in mice.

1. Introduction

Sepsis is defined as a dysregulation of the host response to infection, which leads to a life-threatening organ dysfunction and even multiple organ dysfunction syndrome [1]. Cardiac dysfunction is a fatal complication of sepsis. Several studies have demonstrated that sepsis-induced cardiac dysfunction is observed in 40% of patients with sepsis and is associated with an increased mortality rate up to 70% [2,3]. Therefore, it is important to explore the mechanisms of cardiac dysfunction during sepsis. Sepsis-induced cardiac dysfunction appears to involve complex pathophysiological features, which are characterized by energy depletion, alteration of calcium ion homeostasis, oxidative stress as well as cardiomyocyte death [4,5]. Recently, accumulating studies have revealed that cardiomyocyte apoptosis plays an important role in the pathogenesis of cardiac dysfunction during sepsis [6,7]. Therefore, cardiomyocyte apoptosis is a potential therapeutic target for sepsis-induced cardiac dysfunction.

Autophagy is an intracellular degradation process through which cytosolic constituents, misfolded proteins and damaged organelles are sequestrated into double-membrane vesicles and then delivered to lysosome for degradation [8]. The LC3-I is conjugated with phosphatidylethanolamine to generate LC3-II, which plays a critical role in cargo recruitment, autophagosome biogenesis and completion [9]. LC3-II/LC3-I ratio is regarded as an indicator of autophagic activity. p62 is incorporated into autophagosome by binding to LC3-II and then degraded in the process of autophagy. Thus, p62 is negatively correlated with the autophagy [10]. Accumulating studies have demonstrated that autophagy is essential to maintain cellular homeostasis and improve

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https://doi.org/10.1016/j.lfs.2018.11.055
Received 4 November 2018; Received in revised form 25 November 2018; Accepted 26 November 2018
Available online 27 November 2018
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survival, which has been implicated in the pathogenesis of various diseases [11,12]. Recently, increasing evidence has found that autophagy is involved in the sepsis-induced cardiac dysfunction. The activation of autophagy improved the cardiac function during sepsis [13,14]. However, autophagy deficiency deteriorated sepsis-induced cardiac dysfunction [15]. These studies suggested that autophagy had protective effects against sepsis-induced cardiac dysfunction.

Melatonin is an endogenous indoleamine hormone mainly secreted by the pineal gland and plays a crucial role in the regulation of circadian rhythms [16]. Moreover, numerous studies have demonstrated that melatonin exerts protective role in cardiovascular diseases, including myocardial infarction, hypertension, diabetic cardiomyopathy and heart failure [17]. The beneficial effects of melatonin in cardiovascular diseases are attributed to its anti-inflammation, anti-oxidation and anti-apoptosis properties [18,19]. Recently, several studies have shown that melatonin prevents the development of cardiac dysfunction and improves survival during sepsis [20,21]. However, the underlying mechanisms that melatonin protects against sepsis-induced cardiac dysfunction remain unclear.

Silent information regulator 1 (SIRT1) is a conserved nicotinamide adenine dinucleotide (NAD^+) dependent histone deacetylase and has been implicated in a variety of intracellular signals, such as senescence, inflammation, apoptosis and autophagy [22–25]. Previous studies have demonstrated that SIRT1 was decreased in the myocardium of septic mice and activation of SIRT1 improved the cardiac dysfunction induced by cecal ligation and puncture [26]. Therefore, SIRT1 has been regarded as a target for sepsis-induced cardiac dysfunction. Recently, there is some evidence that melatonin confers protective effects against cardiac dysfunction induced by sepsis [20,21]. In addition, melatonin has been reported to be a potent regulator of SIRT1 in many diseases [27–29]. However, the role of SIRT1 in the protective effects of melatonin against sepsis-induced myocardial dysfunction remains elusive. Therefore, this study was aimed to investigate whether melatonin protected against sepsis-induced cardiac dysfunction by regulating apoptosis and autophagy via activation of SIRT1 in mice with sepsis.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Animal Care and Use Committee of the Second Xiangya Hospital, Central South University (Changsha, China) and were performed in accordance with the National Institutes of Health Guidelines on the Use of Laboratory Animals. Male C57BL/6 mice were purchased from the laboratory animal center of Shanghai SLAC (Shanghai, China). The mice were bred with a 12-hour light/dark cycle pattern and had free access to food and water.

2.2. Experimental protocol

Twenty-four male C57BL/6 mice were randomly assigned to four groups ($n = 6$ each): (1) Control group; (2) LPS group; (3) LPS + Melatonin group; and (4) LPS + Melatonin + EX527 group. Control group was injected intraperitoneally with vehicle. LPS group was injected intraperitoneally with 15 mg/kg LPS (Sigma-Aldrich, St. Louis, USA, Cat. NO. L2880) for 8 h to establish the animal model of sepsis-induced cardiac dysfunction as described previously [30]. LPS + Melatonin group was injected intraperitoneally with 3 doses of 30 mg/kg melatonin (Sigma-Aldrich, St. Louis, USA, Cat. NO. M5250) as follows: the first dose 30 min before treatment with LPS, the second dose 2 h after treatment with LPS and the third dose 4 h after treatment with LPS. LPS + Melatonin + EX527 group was injected intraperitoneally with 5 mg/kg EX527 (Selleck Chemicals, Houston, USA, Cat. NO. S1541), a SIRT1 inhibitor, 1 h before treatment with LPS. The doses of melatonin and EX527 used in this study were based on the previous studies [31,32]. The cardiac function was examined 8 h after LPS treatment. And then the serum and hearts were harvested for further investigation.

2.3. Echocardiography

The cardiac function was assessed by echocardiography 8 h after LPS administration as described previously [33]. Briefly, mice were anesthetized with 10% chloral hydrate and then placed in recumbent position. Echocardiographic images were recorded using the Acuson S3000 imaging system (Siemens, Germany) equipped with a 17-MHz linear transducer. Parameters of cardiac function were measured on the M-mode images, which were obtained from the parasternal short axis view at papillary muscle level. Left ventricle internal diameters at end-diastole and endsystole were measured. The ejection fraction (EF) and fractional shortening (FS) were calculated to assess cardiac function. All measurements were performed by one investigator blinded to the treatment.

2.4. Measurement of CK and CK-MB

Eight hours after LPS administration, mice were anesthetized with 10% chloral hydrate. The blood samples were collected via orbital sinus puncture and stored at room temperature for 1 h. Serum was separated by centrifugation at 3000 rpm for 10 min. The levels of creatine kinase (CK) and creatine kinase-MB (CK-MB) in serum were analyzed using a Beckman LX-20 Fully Automated Biochemistry Analyzer (Beckman, California, USA) according to the manufacturer's instructions.

2.5. Histological analysis

Eight hours after LPS administration, myocardial tissues of various groups were collected and immediately fixed with 4% paraformaldehyde at room temperature for 48 h. The samples were then embedded with paraffin, sectioned transversely into 4 μm thickness and deparaffinized with xylene. Finally, the slides were stained with hematoxylin and eosin and viewed by a light microscope at 400× magnification for histological analysis. The cardiomyocyte cross-sectional areas were assessed by measuring the circumferential length of the cardiomyocyte using Image J software as described previously [34].

2.6. TUNEL staining

The cardiomyocyte apoptosis was determined by TUNEL staining according to the manufacturer's instructions. Briefly, the myocardial tissues of various groups were fixed in 4% paraformaldehyde, embedded with paraffin and sectioned into slides about 4 μm thick. The slides were incubated with proteinase K solution at room temperature for 30 min. And then the slides were added with 50 μl TUNEL reaction mixture (Roche, Basel, Switzerland, Cat. NO. 11684817910) and incubated at 37 °C for 60 min in the dark chamber. After rinsed with PBS for three times, the slides were incubated with DAPI at room temperature for 10 min to detect the nuclei in the darkness. The slides were washed with PBS and then visualized with fluorescence microscopy at 400× magnification. The TUNEL positive cells were labeled with green fluorescence and DAPI positive cells were displayed with blue fluorescence. Apoptotic index was calculated as the ratio of TUNEL positive cells to DAPI positive cells.

2.7. Transmission electron microscopy

The number of autophagosome was detected by transmission electron microscopy in accordance with the previous study [35]. In brief, myocardial tissues were cut into about 1 mm × 1 mm × 1 mm pieces and fixed with 2.5% glutaraldehyde at 4 °C for 2 h. The samples were then immersed in 1% osmium tetroxide for 2 h, dehydrated in a graded

series of ethanol and embedded in epoxy resin. Thereafter, the ultrathin sections were prepared and then stained with uranyl acetate and lead citrate. The autophagosome was observed with transmission electron microscopy (Hitachi HT7700, Tokyo, Japan) and the number of autophagosome was counted.

2.8. Western blot analysis

The protein expressions of SIRT1, cleaved caspase-3, LC3-II/LC3-I ratio, p62 and β-actin were determined by western blot as described previously [36]. Briefly, myocardial tissues of various groups were lysed with RIPA lysis buffer (Beyotime, Shanghai, China) and quantified by the BCA protein assay kit (Beyotime, Shanghai, China). The equal amount of protein in each group was separated by SDS-PAGE gel and then transferred onto PVDF membrane. The membrane was blocked with 5% nonfat milk at room temperature for 1 h and then incubated with the primary antibodies to SIRT1 (Abcam, Cambridge, USA, Cat. NO. ab189494), cleaved-caspase-3 (Abcam, Cambridge, USA, Cat. NO. ab90437), LC3-II (Proteintech, Rosemont, USA, 14600-1-AP), p62 (Proteintech, Rosemont, USA, Cat. NO. 18420-1-AP) or β-actin (Proteintech, Rosemont, USA, Cat. NO. 20536-1-AP) at 4 °C overnight. The membrane was then incubated with the appropriate secondary antibodies at room temperature for 1 h. The protein band was visualized by the ECL kit (Advansta, California, USA, Cat. NO. K-12045-D10) and quantified by Gel-Pro analyzer. The result was expressed as the ratio of target protein versus β-actin. The value of the control group was defined as 100%.

2.9. Statistical analysis

All data were analyzed with SPSS 18.0 and expressed as mean ± SD. Comparisons among multiple groups were performed by one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Melatonin increased SIRT1 protein expression in the myocardium of mice with sepsis

SIRT1 is a therapeutic target for sepsis-induced cardiac dysfunction. To investigate whether SIRT1 is involved in melatonin's protection against cardiac dysfunction during sepsis, we measured SIRT1 protein expression in the myocardium of various groups. As shown in Fig. 1, the protein expression of SIRT1 was significantly decreased in the LPS group as compared with the Control group. However, treatment with melatonin significantly increased the protein expression of SIRT1 as compared with the LPS group. In addition, treatment with SIRT1 inhibitor EX527 significantly decreased SIRT1 protein expression as compared with the LPS + Melatonin group. These data indicated that melatonin increased SIRT1 protein expression in the myocardium of mice with sepsis.

3.2. Melatonin improved cardiac function in mice with sepsis

To investigate whether melatonin improved cardiac function in mice with sepsis, we assessed EF and FS by echocardiography. As shown in Fig. 2, EF and FS were significantly decreased in the LPS group as compared with the Control group. However, treatment with melatonin significantly increased EF and FS as compared with the LPS group, indicating that melatonin improved cardiac function in mice with sepsis. In addition, treatment with SIRT1 inhibitor EX527 significantly abolished melatonin's protection against sepsis-induced cardiac dysfunction. These data indicated that melatonin improved cardiac function in mice with sepsis via SIRT1 pathway.

3.3. Melatonin attenuated myocardial injury in mice with sepsis

CK and CK-MB are enzymes originally existed in the cytoplasm. During myocardial injury, CK and CK-MB are leaked from cardiomyocyte into the blood stream and manifest as biomarkers of myocardial injury. To investigate the effect of melatonin against myocardial injury, we measured CK and CK-MB levels in serum. As shown in Fig. 3, CK and CK-MB levels were significantly increased in the LPS group as compared with the Control group. However, treatment with melatonin significantly decreased CK and CK-MB levels as compared with the LPS group, indicating that melatonin attenuated sepsis-induced myocardial injury. In addition, treatment with SIRT1 inhibitor EX527 significantly abolished melatonin's cardioprotection. These data indicated that melatonin attenuated sepsis-induced myocardial injury via SIRT1 pathway.

3.4. Melatonin attenuated cardiomyocyte cross-sectional areas in mice with sepsis

To investigate the effect of melatonin on myocardial architecture, we measured the cardiomyocyte cross-sectional areas following hematoxylin and eosin staining. As shown in Fig. 4, LPS induced myocardial architecture destruction, as evidenced by the disrupted myocardial fibers and the increased cardiomyocyte cross-sectional areas in the myocardium of the LPS group as compared with the Control group. However, treatment with melatonin significantly decreased the cardiomyocyte cross-sectional areas as compared with the LPS group, indicating that melatonin attenuated myocardial architecture destruction in mice with sepsis. In addition, treatment with SIRT1 inhibitor EX527 significantly increased the cardiomyocyte cross-sectional areas as

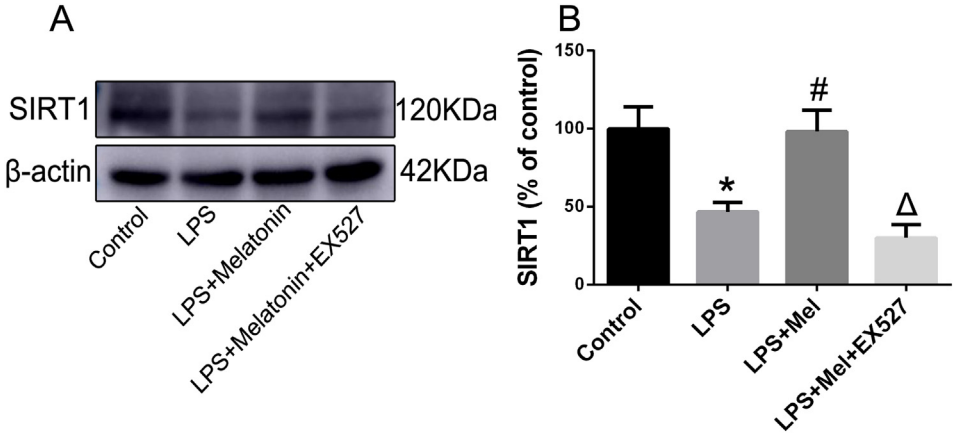

Fig. 1. Melatonin increased SIRT1 protein expression in the myocardium of mice with sepsis. (A) The myocardial tissues were collected for measuring the protein expression of SIRT1 by western blot. (B) The protein expression of SIRT1 was expressed as mean ± SD ($n = 6$). $*P < 0.05$, as compared with the Control group; $\#P < 0.05$, as compared with the LPS group; $\Delta P < 0.05$, as compared with the LPS + melatonin group.

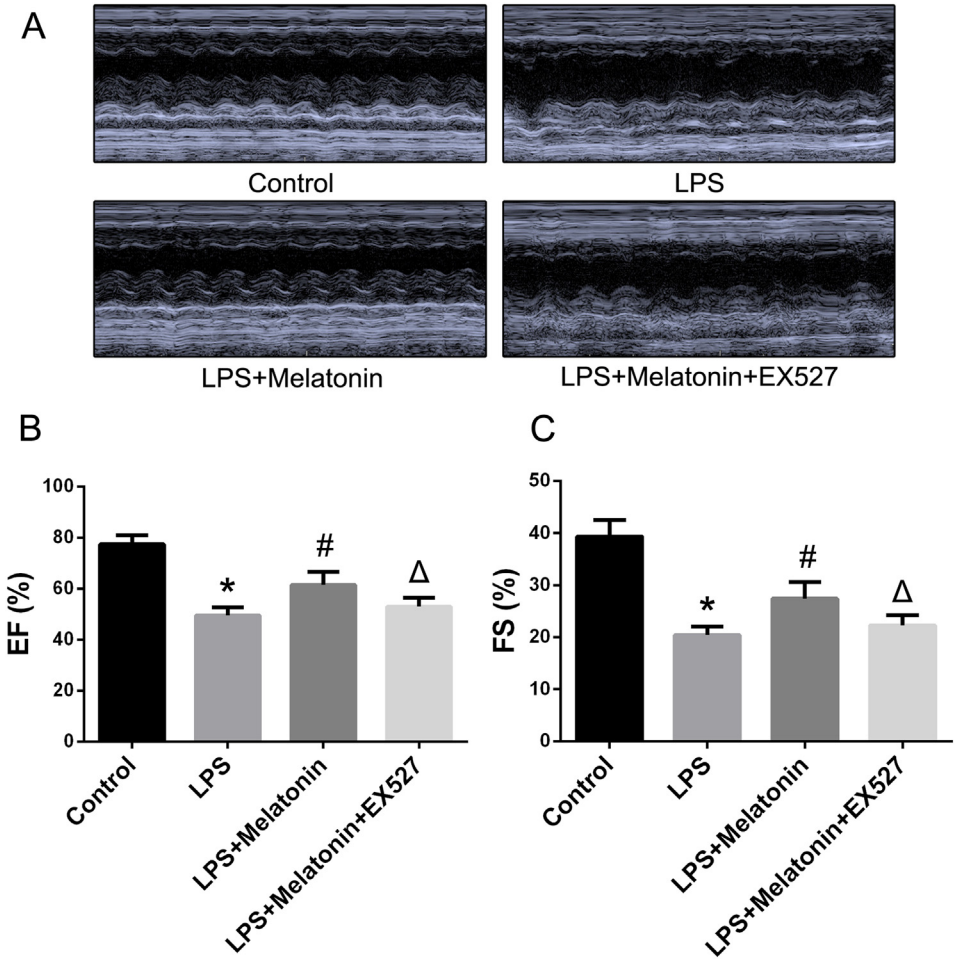

Fig. 2. Melatonin improved cardiac function in mice with sepsis. (A) EF and FS were measured by echocardiography. The data of (B) EF and (C) FS were expressed as mean ± SD ($n = 6$). *$P < 0.05$, as compared with the Control group; #$P < 0.05$, as compared with the LPS group; Δ$P < 0.05$, as compared with the LPS + melatonin group.

compared with the LPS + Melatonin group. These data indicated that melatonin significantly attenuated myocardial architecture destruction in mice with sepsis via SIRT1 pathway.

3.5. Melatonin attenuated apoptosis in the myocardium of mice with sepsis

The cardiomyocyte apoptosis plays an important role in the pathogenesis of cardiac dysfunction during sepsis. To investigate the effect of melatonin on cardiomyocyte apoptosis, we measured the apoptotic index by TUNEL staining and the cleaved caspase-3 protein

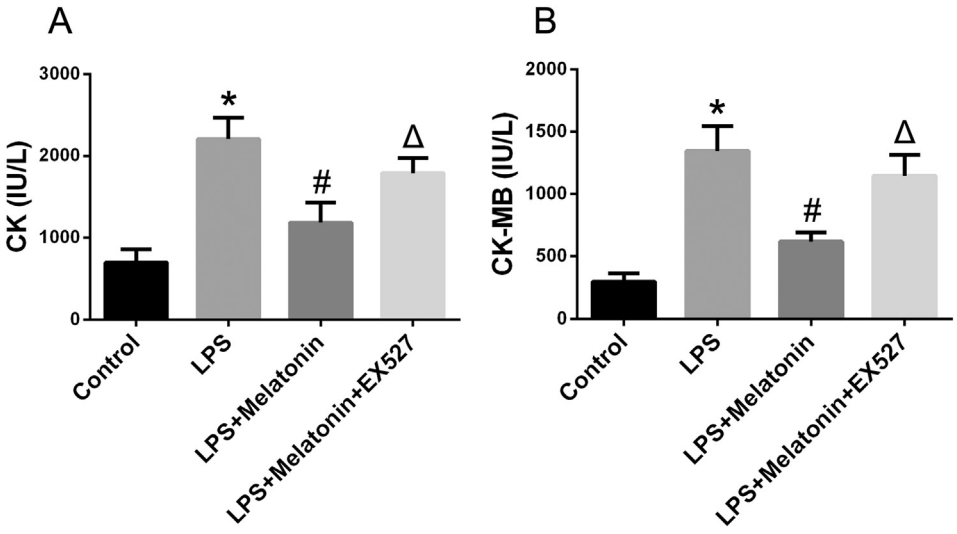

Fig. 3. Melatonin attenuated myocardial injury in mice with sepsis. The levels of CK and CK-MB in serum were detected by a Fully Automated Biochemistry Analyzer. The data of (A) CK and (B) CK-MB were expressed as mean ± SD ($n = 6$). *$P < 0.05$, as compared with the Control group; #$P < 0.05$, as compared with the LPS group; Δ$P < 0.05$, as compared with the LPS + melatonin group.

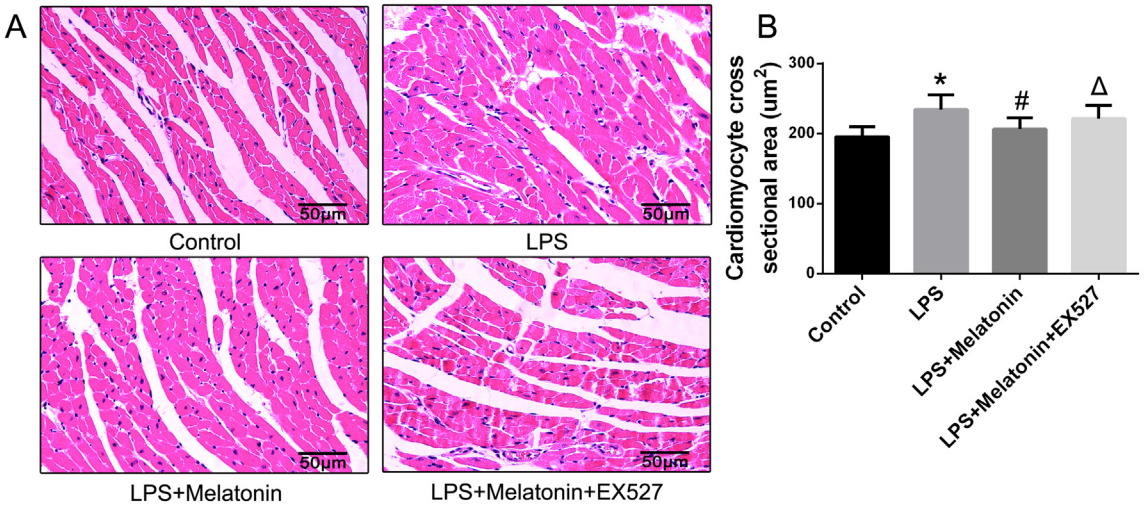

Fig. 4. Melatonin attenuated cardiomyocyte cross-sectional areas in mice with sepsis. (A) The myocardial tissues were stained with hematoxylin and eosin and viewed under the light microscope. (B) The cardiomyocyte cross-sectional areas were measured to assess the extent of myocardial architecture destruction. Data were expressed as mean ± SD ($n = 6$). *$P < 0.05$, as compared with the Control group; #$P < 0.05$, as compared with the LPS group; Δ$P < 0.05$, as compared with the LPS + melatonin group.

expression by western blot in the myocardium of various groups. As shown in Fig. 5, the apoptotic index and the cleaved caspase-3 protein expression were significantly increased in the myocardium of mice with sepsis as compared with the Control group. However, treatment with melatonin significantly attenuated apoptotic index in the myocardium as compared with the LPS group. In addition, the effect of melatonin against apoptosis was abolished by treatment with SIRT1 inhibitor EX527. These data indicated that melatonin attenuated apoptosis in the myocardium of mice with sepsis via SIRT1 pathway.

3.6. Melatonin increased autophagosome in the myocardium of mice with sepsis

Autophagosome is a double-membraned vacuole containing undigested cytoplasm and organelles. To investigate the effect of melatonin on autophagosome, we observed the number of autophagosomes using transmission electron microscope in the myocardium of various groups. As shown in Fig. 6, the number of autophagosomes in the myocardium was significantly increased in the LPS group as compared with the Control group. Moreover, treatment with melatonin further increased the number of autophagosomes as compared with the LPS group. In addition, treatment with SIRT1 inhibitor EX527 significantly decreased the number of autophagosomes as compared with the LPS + Melatonin group. These data indicated that melatonin significantly increased autophagosome in the myocardium of mice with sepsis via SIRT1 pathway.

3.7. Effects of melatonin on LC3-II/LC3-I ratio and p62 protein expression in the myocardium of mice with sepsis

The conversion of LC3-I to LC3-II is the initial step of autophagy and LC3-II/LC3-I ratio is regarded as an indicator of autophagic activity. p62 protein expression level is negatively correlated with autophagy. To investigate the effects of melatonin on autophagic signaling, we measured LC3-II/LC3-I ratio and p62 protein expression in the myocardium of various groups. As shown in Fig. 7, LC3-II/LC3-I ratio was significantly increased, whereas p62 protein expression was significantly decreased in the LPS group as compared with the Control group. Moreover, treatment with melatonin further increased LC3-II/LC3-I ratio and decreased p62 protein expression as compared with the LPS group. In addition, these effects conferred by melatonin were abolished by treatment with SIRT1 inhibitor EX527. These data indicated that melatonin increased autophagy in the myocardium of mice with sepsis via SIRT1 pathway.

4. Discussion

The sepsis-induced cardiac dysfunction is associated with high mortality in patients with sepsis [37]. Compelling evidence has demonstrated that apoptosis and autophagy are involved in the pathogenesis of sepsis-induced cardiac dysfunction [6,7,13]. Recently, several studies have revealed that melatonin exerted protective effects against sepsis-induced cardiac dysfunction [20,21]. However, the underlying mechanisms remain to be elucidated. In this study, we found that melatonin improved cardiac function by regulating apoptosis and autophagy in mice with sepsis. Moreover, we also demonstrated that melatonin increased the expression of SIRT1 in the myocardium of mice with sepsis, while inhibition of SIRT1 by EX527 abolished the protective effects of melatonin against sepsis-induced cardiac dysfunction. Therefore, these findings indicated that melatonin protected against sepsis-induced cardiac dysfunction by regulating apoptosis and autophagy via activation of SIRT1 in mice.

Melatonin is essential for the regulation of sleep homeostasis, circadian rhythms and behavioral modulation [16]. Recently, increasing evidence has demonstrated that melatonin plays an important role in the pathogenesis of sepsis. Lorente et al. found that serum melatonin level was associated with sepsis severity and with 30-day mortality [38]. Moreover, numerous studies reported that melatonin administration protected against organ dysfunction and improved survival rates in animal model of sepsis [39,40]. In addition, several studies also revealed that melatonin had beneficial effects against sepsis-induced cardiac dysfunction [20,21]. Consistent with the previous findings, our results demonstrated that treatment with melatonin significantly increased EF and FS, whereas significantly decreased CK, CK-MB and cardiomyocyte cross-sectional areas as compared with the LPS group, indicating that melatonin improved cardiac function and attenuated myocardial injury in mice with sepsis. However, the underlying mechanisms have not been completely clarified. Previous studies demonstrated that cardiomyocyte apoptosis was observed in the myocardium during sepsis and was implicated in the pathogenesis of sepsis-induced cardiac dysfunction [6,7]. In this study, we also demonstrated that treatment with melatonin significantly decreased cardiomyocyte apoptosis, as evidenced by attenuating apoptotic index and the protein expression of cleaved caspase-3 in the myocardium as compared with

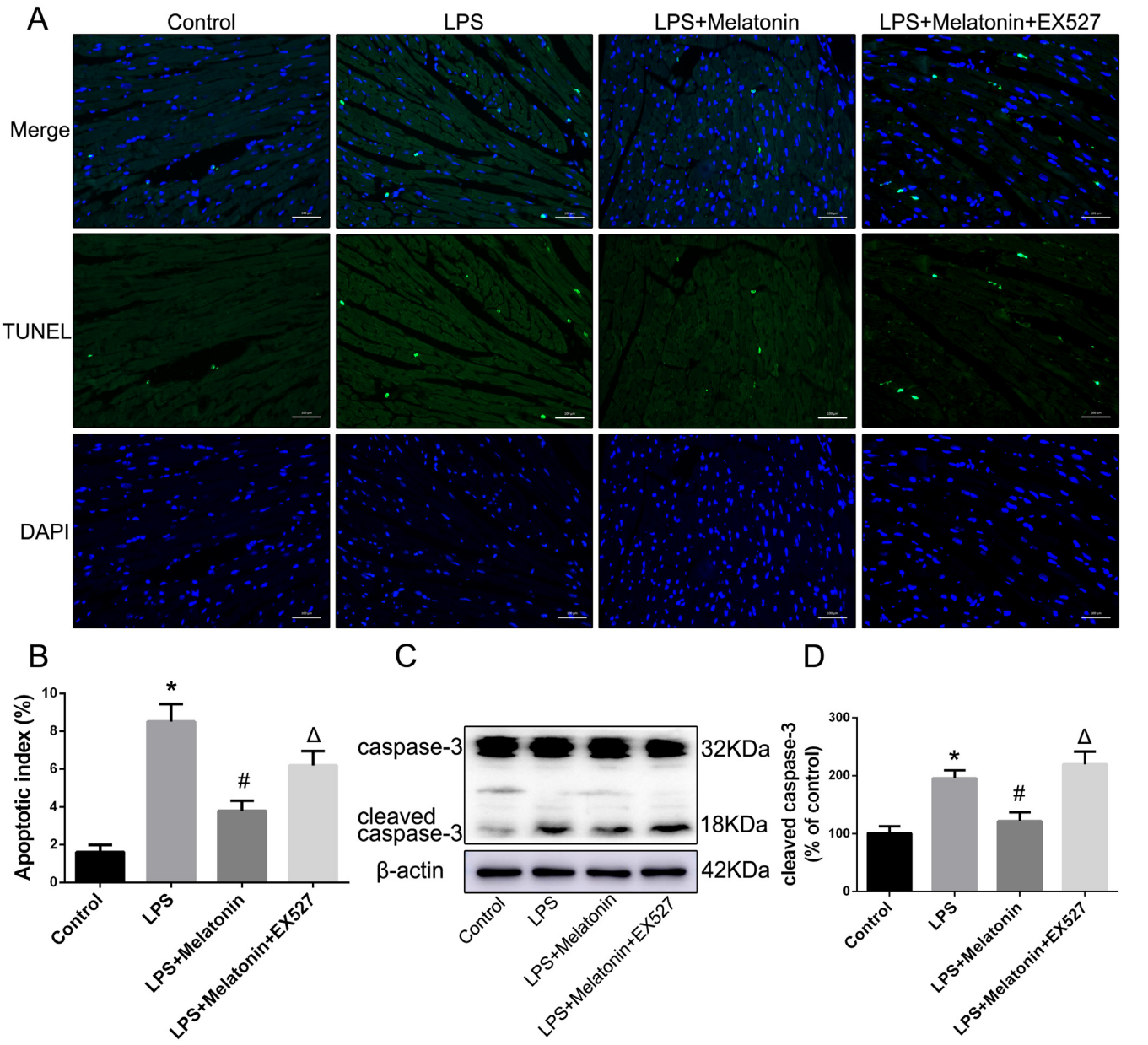

Fig. 5. Melatonin attenuated apoptosis in the myocardium of mice with sepsis. (A) The apoptotic cells were detected by TUNEL (green) and the nuclei were detected by DAPI (blue). (B) Apoptotic index was calculated as the ratio of TUNEL positive cells to DAPI positive cells. (C) The myocardial tissues were collected for measuring cleaved caspase-3 protein expression by western blot. (D) The protein expression of SIRT1 was expressed as mean ± SD ($n = 6$). $*P < 0.05$, as compared with the Control group; $\#P < 0.05$, as compared with the LPS group; $\Delta P < 0.05$, as compared with the LPS + melatonin group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the LPS group. All these results indicated that melatonin protected against sepsis-induced cardiac dysfunction possibly via attenuating cardiomyocyte apoptosis.

Autophagy is a highly conserved intracellular self-digestive process and plays an important role in maintaining cellular homeostasis and promoting cell survival via degrading the damaged organelles and protein aggregates [8]. There is ample evidence that autophagy is essential for the maintenance of cardiac structure and function at baseline or in response to stress [11]. Recently, accumulating findings demonstrated that autophagy was activated in the septic heart [13] or the cultured cardiomyocytes treated with LPS [41]. Moreover, enhancing autophagy protected against sepsis-induced cardiac dysfunction by improving cardiomyocyte contractile and intracellular calcium ion handling in murine challenged with LPS [14]. In addition, inhibition of autophagy abolished the beneficial effects of melatonin against sepsis-induced cardiac dysfunction [15]. These studies indicated that autophagy was a therapeutic target for sepsis-induced cardiac dysfunction. Recently, melatonin has been reported to have protective effects in cardiovascular diseases including ischemic cardiomyopathy, diabetic cardiomyopathy and cardiac hypertrophy by modulation of autophagy [42]. However, whether melatonin attenuates sepsis-induced cardiac dysfunction by regulating autophagy has not been studied. In this study, we found that autophagy was significantly enhanced in the septic heart, as evidenced by upregulating the number of autophagosomes and LC3-II/LC3-I ratio, and downregulating p62 in the myocardium of mice with sepsis as compared with the Control group, which is consistent with previous studies. Moreover, we also demonstrated that treatment with melatonin further increased autophagy in the myocardium as compared with the LPS group, which contributed to the protective effects of melatonin against sepsis-induced cardiac dysfunction.

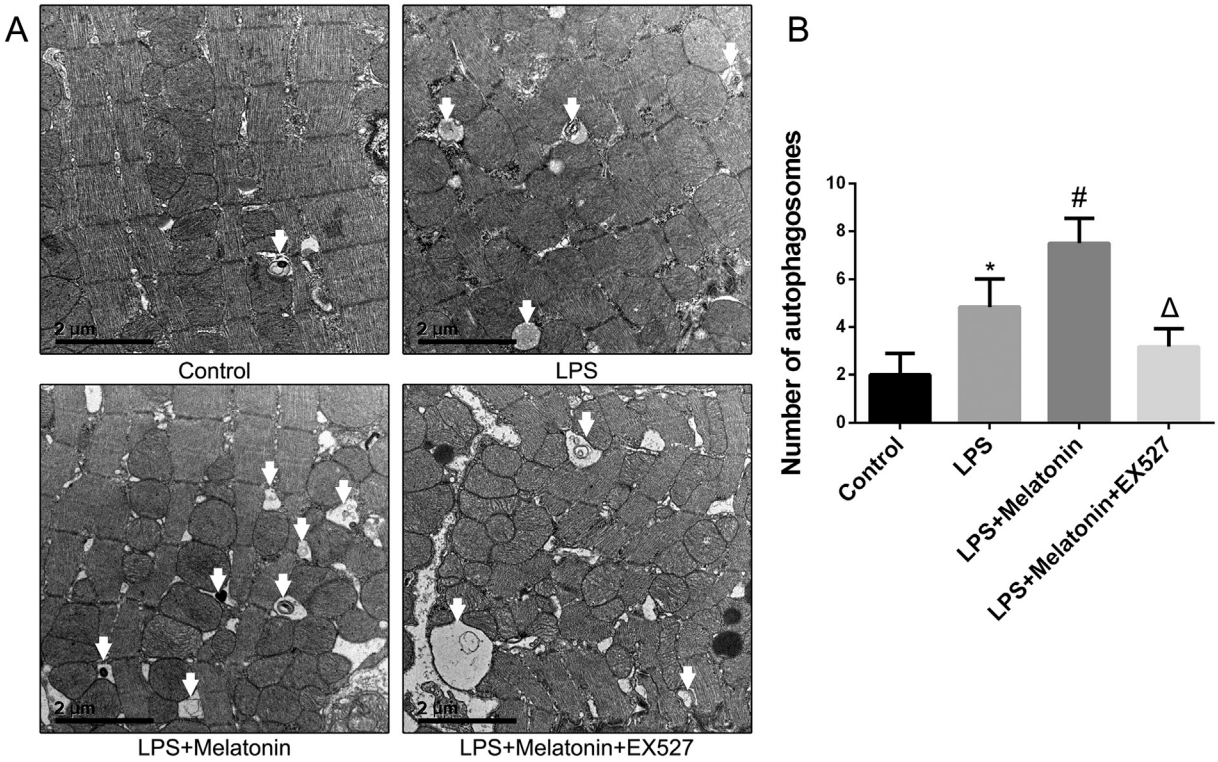

Fig. 6. Melatonin increased autophagosome in the myocardium of mice with sepsis. (A) The autophagosome was observed by transmission electron microscope. (B) The number of autophagosomes in the myocardium was expressed as mean ± SD ($n = 6$). $*P < 0.05$, as compared with the Control group; $\#P < 0.05$, as compared with the LPS group; $\Delta P < 0.05$, as compared with the LPS + melatonin group.

SIRT1 was reported to be involved in the pathogenesis of sepsis-induced cardiac dysfunction. Han et al. demonstrated that SIRT1 was significantly decreased in the myocardium of mice subjected to cecal ligation and puncture, and pharmacological activation of SIRT1 protected against sepsis-induced cardiac dysfunction and improved mice survival [26]. Therefore, SIRT1 might be a novel therapeutic target for sepsis-induced myocardial dysfunction. Recently, melatonin has been reported to induce the activation of SIRT1 in many diseases [27–29]. However, whether SIRT1 is involved in the protective effects of melatonin against sepsis-induced myocardial dysfunction remains unclear. In this study, we found that treatment with melatonin significantly increased SIRT1 protein expression in the myocardium of mice with sepsis, while inhibition of SIRT1 by EX527 abolished melatonin's cardioprotection during sepsis, suggesting that activation of SIRT1 was required for the protective effects of melatonin against sepsis-induced cardiac dysfunction. Numerous studies also demonstrated that SIRT1 attenuated myocardial injury by inhibiting apoptosis and activating autophagy in many conditions [35,43]. In this study, we also found that treatment with SIRT1 inhibitor EX527 significantly increased apoptosis and decreased autophagy in the myocardium of LPS + Melatonin + EX527 group as compared with the LPS + Melatonin group. All these results suggested that melatonin protected against sepsis-induced cardiac dysfunction by regulating apoptosis and autophagy via activation of SIRT1 in mice with sepsis. However, whether melatonin protects against cardiac dysfunction in patients with sepsis needs for further clinical trials.

In conclusion, our results demonstrated that melatonin had beneficial effects against cardiac dysfunction induced by sepsis. The cardioprotective effects of melatonin were possibly attributed to inhibiting apoptosis and activating autophagy via activation of SIRT1 in mice with

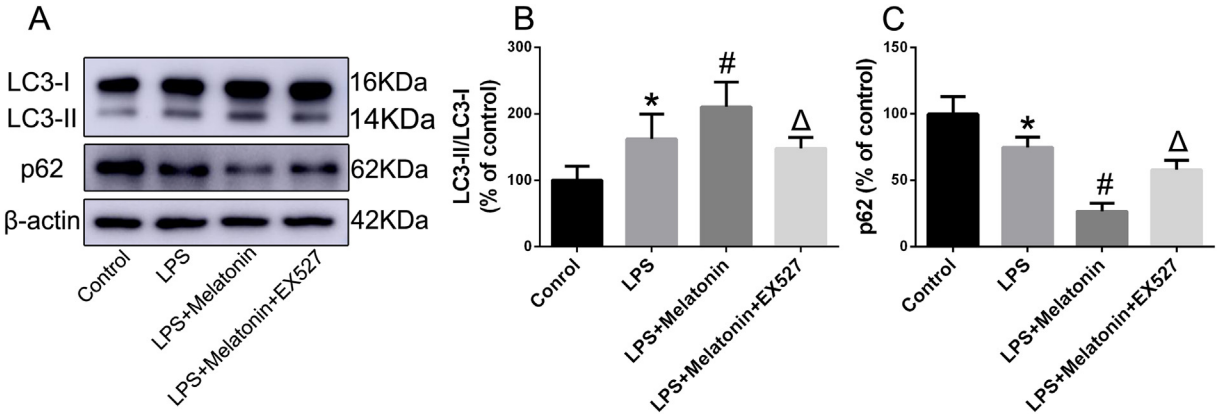

Fig. 7. Effects of melatonin on LC3-II/LC3-I ratio and p62 protein expression in the myocardium of mice with sepsis. (A) The myocardial tissues were collected for measuring LC3-II/LC3-I ratio and p62 protein expression by western blot. (B) LC3-II/LC3-I ratio and (C) p62 protein expression were expressed as mean ± SD ($n = 6$). $*P < 0.05$, as compared with the Control group; $\#P < 0.05$, as compared with the LPS group; $\Delta P < 0.05$, as compared with the LPS + melatonin group.

sepsis. Therefore, our findings provide evidence that melatonin is a promising therapeutic target for sepsis-induced cardiac dysfunction.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81100221), Fundamental Research Funds for the Central Universities (2011QNZT175) and Specialized Research Fund for the Doctoral Program of Higher Education of China (20110162120012).

Conflict of interest

The authors have no conflict of interest.

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