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ORIGINAL ARTICLE

Polydatin Alleviates Septic Myocardial Injury by Promoting SIRT6-Mediated Autophagy

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Abstract— Sepsis is a life-threatening condition. Polydatin (PD), a small natural compound from *Polygonum cuspidatum*, possesses antioxidant and anti-inflammatory properties. However, the protective mechanism of PD on sepsis-induced acute myocardial damage is still unclear. The aim of this study was to investigate the effect and mechanism of action of PD on lipopolysaccharide (LPS)-induced H9c2 cells and in a rat model of sepsis, and explored the role of PD-upregulated sirtuin (SIRT)6. LPS-induced H9c2 cells were used to simulate sepsis. Cecal ligation and puncture (CLP)-induced sepsis in rats were used to verify the protective effect of PD. ELISA, western blotting, immunofluorescence, immunohistochemistry, and flow cytometry were used to study the protective mechanism of PD against septic myocardial injury. PD pretreatment suppressed LPS-induced H9c2 cell apoptosis by promotion of SIRT6-mediated autophagy. Downregulation of SIRT6 or inhibition of autophagy reversed the protective effect of PD on LPS-induced apoptosis. PD pretreatment also suppressed LPS-induced inflammatory factor expression. CLP-induced sepsis in rats showed that PD pretreatment decreased CLP-induced myocardial apoptosis and serum tumor necrosis factor-α, interleukin (IL)-1β, and IL-6 expression. 3-Methyladenine (autophagy inhibitor) pretreatment prevented the protective effect of PD on septic cardiomyopathy. SIRT6 expression was increased with PD treatment, which confirmed that PD attenuates septic cardiomyopathy by promotion of SIRT6-mediated autophagy. All these results indicate that PD has potential therapeutic effects that alleviate septic myocardial injury by promotion of SIRT6-mediated autophagy.

KEY WORDS: polydatin; myocardial injury; sepsis; autophagy; SIRT6.

INTRODUCTION

Severe sepsis and septic shock are major healthcare problems throughout the world annually [1]. Multiple organ failure in sepsis is a consequence of cellular dysfunction in the affected organs [2, 3], and there is compelling evidence that cardiovascular dysfunction is a major complication associated with sepsis-induced morbidity and mortality [4]. Polydatin (PD), a glucoside of resveratrol, known as a monocrystalline compound isolated from *Polygonum cuspidatum Sieb. et Zucc.*, is used for both medication and food [5]. Increasing evidence shows that PD has strong anti-inflammatory and antioxidative bioactivity

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[6–8]. PD inhibits mitochondrial dysfunction in the renal tubular epithelial cells of a rat model of sepsis-induced acute kidney injury [9]. PD also has protective effects on septic lung injury in mice *via* upregulation of heme oxygenase-1 [10]. Whether PD has a protective effect on sepsis-induced acute myocardial damage is still unclear.

Autophagy is a cellular degradation process that is distinct from the ubiquitin–proteasome system, and is a primary mechanism for maintaining cellular homeostasis. Increasing evidence shows a relationship between autophagy and apoptosis [11–13]. Some studies have found that autophagy plays an important role in cell survival after sepsis [14, 15]. Previous studies have been found that valproic acid can attenuate sepsis-induced myocardial dysfunction in rats by accelerating autophagy through the PTEN/AKT/mTOR pathway [16]. MiR-155 alleviates septic lung injury by inducing autophagy *via* inhibition of transforming growth factor-β-activated binding protein 2 [17]. However, excessive autophagy can cause unwanted and deleterious cell death [18]. PD regulates cell proliferation, apoptosis, and autophagy [19], but the specific mechanism is not clear.

Sirtuin (SIRT)6, which belongs to the sirtuin family of NAD^+-dependent enzymes, has many pivotal functions and exhibits multiple enzymatic activities [20]. Expression of SIRT6 promotes activation of autophagy [21, 22]. Previous studies have found that sirt6-induced autophagy restricted TREM-1-mediated pyroptosis in ox-LDL-treated endothelial cells: relevance to prognostication of patients with acute myocardial infarction [23]. PD ameliorates diabetic cardiomyopathy *via* SIRT3 activation [24]. Both SIRT3 and SIRT6 belong to the NAD^+-dependent class III deacetylase sirtuin family [25]. It is suggested that PD alleviates septic myocardial injury by promoting SIRT6-mediated autophagy.

In the present study, we investigated the function and potential mechanism of action of PD on sepsis-mediated cardiomyopathy and elucidated the protective mechanism of PD through a cecal ligation and puncture (CLP) rat model and lipopolysaccharide (LPS)-induced H9c2 cell damage.

MATERIALS AND METHODS

Ethics Statement

All animal experiments were approved by the Animal Ethics Committee of Pudong New Area Gongli Hospital (Shanghai, China).

Sepsis Model and Treatment

Male Sprague-Dawley rats (age 7–8 weeks and weighing 200–250 g) were provided by Shanghai SLAC Laboratory Animal Co. (Shanghai, China). Rats were provided with irradiated food, free access to sterile acidified water, and were housed in individual microisolators. For sepsis induction, animals were anesthetized by intraperitoneal injection of 2% sodium pentobarbital in saline (40 mg/kg; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and placed on a warming pad (37 °C). CLP was performed as previous studies [9]. The rat abdomen was shaved before a 2-cm midline abdominal incision was made. To prevent bowel obstruction, the cecum was carefully isolated, and ~60% of the total cecum length was ligated below the ileocecal valve. Two punctures were made on the opposite side of the mesenteric with a sterile 18-gauge needle. The cecum was gently pressed to expel a small amount of stool from the puncture site to ensure a full-thickness perforation. The cecum was returned to the peritoneal cavity, and the abdominal incision was closed in two layers. The ceca of sham-operated rats were not ligated or punctured, but underwent the same aforementioned procedure. All rats were subcutaneously injected with normal saline (5 ml/100 g) immediately following surgery in order to resuscitate. They were then group-housed in a temperature-controlled room (22 °C) in cages with dry sawdust bedding. Fluid blocks and soft food were provided. The mortality and behavioral signs were monitored and recorded every day following the CLP procedure.

To investigate the effect of PD and autophagy on sepsis-induced cardiomyopathy, PD (30 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) [9] or the autophagic inhibitor, 3-methyladenine (3-Ma, 20 mg/kg; Sigma-Aldrich) was administered intraperitoneally 6 h before CLP. An equal dose of normal saline was administered to the control group. Each group comprised ten rats. At 48 h after surgery, the hearts were collected for subsequent experiments after statistical analysis of survival.

Echocardiographic and Hemodynamic Measurements

We anesthetized rats with intramuscular injections of ketamine (100 mg/kg) and medetomidine (0.25 mg/kg). Cardiac function parameters such as left ventricular internal dimension in systole (LVID(s)), left ventricular internal dimension in diastole (LVID(d)), left ventricular fraction shortening (LVFS), and left ventricular ejection fraction (LVEF) were captured by transthoracic echocardiography system, which had a 15-MHz phased-array transducer (SONOS 5500; Hewlett-Packard, Andover, MA, USA).

We inserted a catheter into the rat left ventricle from the right carotid artery. In this manner, the left ventricular end diastolic pressure (LVEDP), heart rate (HR), and cardiac contractility (dP/dt maximum and dP/dt minimum) were assayed and recorded with a PowerLab ML880 (AD Instruments, Bella Vista, NSW, Australia).

Cell Lines and Cell Culture

The cardiomyoblast cell line H9c2 was purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. To investigate whether SIRT6 plays a role in sepsis-mediated myocardial protection and to clarify its mechanism of action, H9c2 cells were transfected with siRNA against SIRT6 (constructed by Genechem, Shanghai, China) for 48 h before induction with LPS (1 μg/ml) for 24 h. H9c2 cells were harvested for biological analysis. To investigate the role of autophagy in sepsis-mediated myocardial damage, H9c2 cells transfected with GFP-LC3 vector (Genechem, Shanghai, China) were pretreated with PD (100 μM) or 3-Ma (10 μM) for 30 min before induction with LPS (1 μg/ml) for 24 h.

Immunofluorescence and Immunohistochemistry

The myocardial sections and H9c2 cells were deparaffinized and heated in citrate buffer for antigen retrieval. Phosphate-buffered saline containing 10% fetal bovine serum was added to each section to reduce background staining for 30 min at room temperature. Sections of 5 μm were used for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining for apoptosis analysis with an Axiophot light microscope (Zeiss, Oberkochen, Germany). For autophagy analysis, H9c2 cells were stained with LC3 antibody and analyzed by fluorescence microscopy (Nikon, Tokyo, Japan) and photographed with a digital camera.

Flow Cytometry

Flow cytometry was used to determine the rate of apoptosis of H9c2 cells. Apoptotic cells were differentiated from viable or necrotic cells by the combined application of annexin V (AV)–fluorescein isothiocyanate (FITC) and propidium iodide (PI). Cells were washed twice and adjusted to a concentration of 10^6 cells/ml with cold D-Hanks buffer. AV–FITC (10 μl) and PI (10 μl) were added to 100 μl of cell suspension and incubated for 15 min at room temperature in the dark. Finally, 400 μl of binding buffer was added to each sample without washing and analyzed using flow cytometry. Each experiment was performed at least in three independent experiments.

Western Blot Analysis

Cells or tissues were lysed; protease inhibitors were added to the lysates; and cell lysate was centrifuged at 12000 rpm at 4 °C. The protein concentration was examined by the BCA kit (Pierce, Rockford, IL, USA). The protein was separated using 10% SDS-PAGE assay and moved to a PVDF membrane. Antibodies listed below were applied to determine the protein expression: SIRT6 (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), cleaved caspase-3 (1:1000; Santa Cruz Biotechnology), caspase-3 (1:1000; Santa Cruz Biotechnology), Beclin-1 (1:500; Santa Cruz Biotechnology), P62 (1:500; Santa Cruz Biotechnology), LC3 (1:500; Santa Cruz Biotechnology), Bcl-2 (1:500; Santa Cruz Biotechnology), Bax (1:1000; Santa Cruz Biotechnology), GAPDH (1:1000; Santa Cruz Biotechnology), and horseradish-peroxidase-conjugated secondary antibody (1:1000; Abcam, Cambridge, MA, USA). An ECL chemiluminescent kit (Millipore, Billerica, MA, USA) was applied to measure the bands.

ELISA for Soluble Inflammatory Cytokines

The amounts of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in the supernatants of H9c2 cells or serum from rats were measured using commercially available ELISA kits (Sen-Xiong Company). Supernatants were stored at − 80 °C before measurement, and both standards and samples were run in triplicate. OD_{450} was calculated by subtracting the background, and standard curves were plotted.

Statistical Analysis

All data are expressed as the mean ± SEM. Data analysis was performed using GraphPad Prism 5.0 (GraphPad, La Jolla, CA, USA). When analyzing two groups, Student's t test was used to determine statistical significance. When comparing multiple groups, one-way analysis of variance was used, followed by Tukey's multiple comparisons test to determine statistically significant differences. $P < 0.05$ was considered significant.

RESULTS

PD Pretreatment Attenuated LPS-Induced Inflammation and Apoptosis of H9c2 Cells by Promotion of Autophagy

PD is a monocrystalline and polyphenolic drug isolated from a traditional Chinese herb (*P. cuspidatum*). It is protective against cell dysfunction induced by inflammatory factors and has been approved for clinical trials for treatment of diabetic cardiomyopathy [24] and myocardial infarction [26, 27]. The mechanism of the protective effects of PD on sepsis-induced acute myocardial damage is still unclear. H9c2 cells were used to study the protective effect of PD on LPS-induced myocardial cell damage. H9c2 cell apoptosis was increased after simulation of sepsis conditions with LPS (1 μg/ml) for 24 h. PD pretreatment decreased LPS-induced apoptosis. Inhibition of autophagy with 3-Ma pretreatment reversed the protective effect of PD on LPS-induced H9c2 cell apoptosis (Fig. 1a, b). Western blotting showed that PD pretreatment downregulated LPS-induced caspase-3 and Bax expression but promoted Bcl-2 expression. 3-Ma pretreatment suppressed PD-induced Bcl-2 expression and promoted cleaved caspase-3 and Bax expression (Fig. 1c–f). ELISA showed that PD pretreatment decreased LPS-induced IL-1β, IL-6, and TNF-α expression. The inhibitory effects of PD were reversed by pretreatment with 3-Ma (Fig. 1g–i). It is suggested that autophagy is involved in the PD-mediated protective effect against LPS-induced H9c2 cell damage, which was confirmed by immunofluorescence. PD pretreatment promoted LPS-induced autophagy plaque formation (Fig. 1j, k). Western blotting showed that PD pretreatment promoted LPS-induced, autophagy-related protein LC3-II and Beclin-1 expression, but decreased P62 expression (Fig. 1l–o).

Downregulation of SIRT6 Reversed the Suppressive Effect of PD on LPS-Induced Inflammation and Apoptosis of H9c2 Cells by Inhibition of Autophagy

Increasing evidence shows that SIRT6 overexpression has a protective effect against stress-induced apoptosis [22]. To confirm whether SIRT6 was involved in PD-mediated protection against LPS-induced H9c2 cell damage, siRNA against SIRT6 was constructed. RT-PCR and western blotting showed that SIRT6 expression was downregulation after SIRT6 silencing when compared with control or negative control groups (Fig. 2a, b). Apoptosis was analyzed by flow cytometry, which showed that downregulation SIRT6 suppressed the protective effect of PD on survival of H9c2 cells treated with LPS condition (Fig. 2c, d). Western blotting confirmed that silencing SIRT6 decreased the inhibitory effect of PD on cleaved caspase-3 and Bax expression. Downregulation of SIRT6 expression also decreased Bcl-2 expression, even with PD pretreatment (Fig. 2e–h). ELISA showed that downregulation of SIRT6 expression reversed PD-induced inhibition of inflammatory factor IL-1β, IL-6, and TNF-α expression under LPS treatment (Fig. 2i–k).

To confirm that SIRT6 had a regulatory effect on autophagy, immunofluorescence showed that PD pretreatment promoted autophagy under LPS treatment. Silencing of SIRT6 suppressed autophagy, even with PD pretreatment (Fig. 3a, b). Western blotting confirmed that downregulation of SIRT6 suppressed Beclin-1 expression and reduced the ratio of LC3-II/LC3-I. Silencing SIRT6 promoted P62 expression, which had inhibitory effect on autophagy (Fig. 3c–f).

PD Pretreatment Attenuated Sepsis-Induced Myocardial Apoptosis and Ameliorated Survival in Rats

To establish whether PD had a protective effect against sepsis, PD (30 mg/kg) or 3-Ma (20 mg/kg) was administered intraperitoneally 6 h before CLP. Each group had 6 rats. After CLP, echocardiography showed significantly decreased LVFS and LVEF, while LVID significantly increased compared with sham rats; importantly, these changes were partially alleviated by PD treatment. However, after autophagy activation suppressed with 3-Ma treatment, the protective effect of PD was reversed. Hemodynamic examination demonstrated that CLP-mediated sepsis decreased dP/dt and increased LVEDP, which were both ameliorated by PD treatment; however, after autophagy activation suppressed with 3-Ma treatment, the protective effect of PD was reversed (Table 1).

Immunohistochemical staining showed that myocardial apoptosis was increased after CLP for 48 h when compared with normal controls. After PD pretreatment, myocardial apoptosis was decreased compared with the CLP group. 3-Ma pretreatment decreased the protective effect of PD against CLP-induced myocardial damage (Fig. 4a, b). PD pretreatment decreased sepsis-induced myocardial apoptosis by promotion of autophagy. Western blotting confirmed that PD pretreatment suppressed sepsis-induced cleaved caspase-3 and Bax expression in

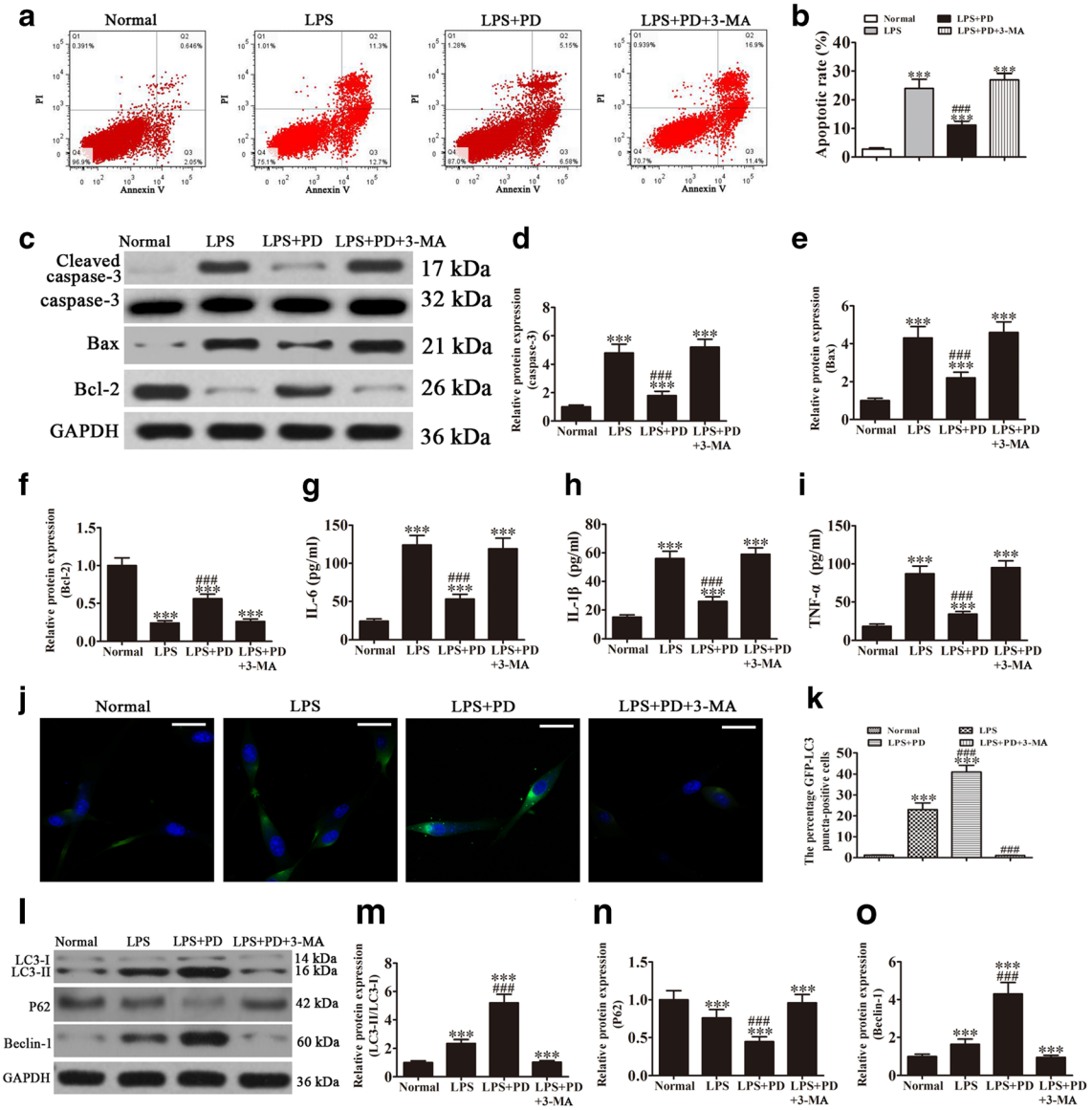

Fig. 1. PD treatment attenuated LPS-induced inflammation and apoptosis of H9c2 cells by promotion of autophagy. H9c2 cells were treated with LPS (1 μg/ml) after pretreatment with PD (100 μM) combined with or without autophagy inhibitor 3-MA (10 μM) for 24 h. **a** Apoptosis was analyzed by flow cytometry after double labeling with AV–FITC and PI. **b** the percentage of apoptotic cells in each group was analyzed. Data are presented as the mean ± SD. $^{***}P < 0.001$ *versus* normal control. $^{###}P < 0.001$ *versus* LPS treatment group. **c**–**f** Western blotting showed expression of cleaved caspase-3, caspase-3, Bcl-2, and Bax. GAPDH was used as an endogenous control. Data are presented as the mean ± SD. $^{***}P < 0.001$ *versus* the normal controls. $^{###}P < 0.001$ *versus* LPS treatment group. **g**–**i** inflammatory factors IL-1β, IL-6, and TNF-α from the supernatant were detected by ELISA. Data are presented as the mean ± SD. $^{***}P < 0.001$ *versus* normal controls. $^{###}P < 0.001$ *versus* LPS treatment group. **j** and **k** Immunofluorescence showed autophagy puncta in H9c2 cells. Scale bar, 20 μm. Data presented as mean ± SD. $^{***}P < 0.001$ *versus* controls. $^{###}P < 0.001$ *versus* LPS treatment group. **l**–**o** Western blotting showed expression of P62, LC3, and Beclin-1. Data presented as mean ± SD. $^{***}P < 0.001$ *versus* normal controls. $^{###}P < 0.001$ *versus* LPS treatment group.

myocardial tissue. PD pretreatment promoted Bcl-2 expression (Fig. 4c–f).

Mortality was monitored from 4 to 48 h before the rats were killed. Mortality of rats with CLP-induced sepsis was increased. PD pretreatment suppressed death after CLP. Pretreatment with 3-Ma decreased the protective effect of PD against CLP-induced death of rats in the acute phase of sepsis (Fig. 4g).

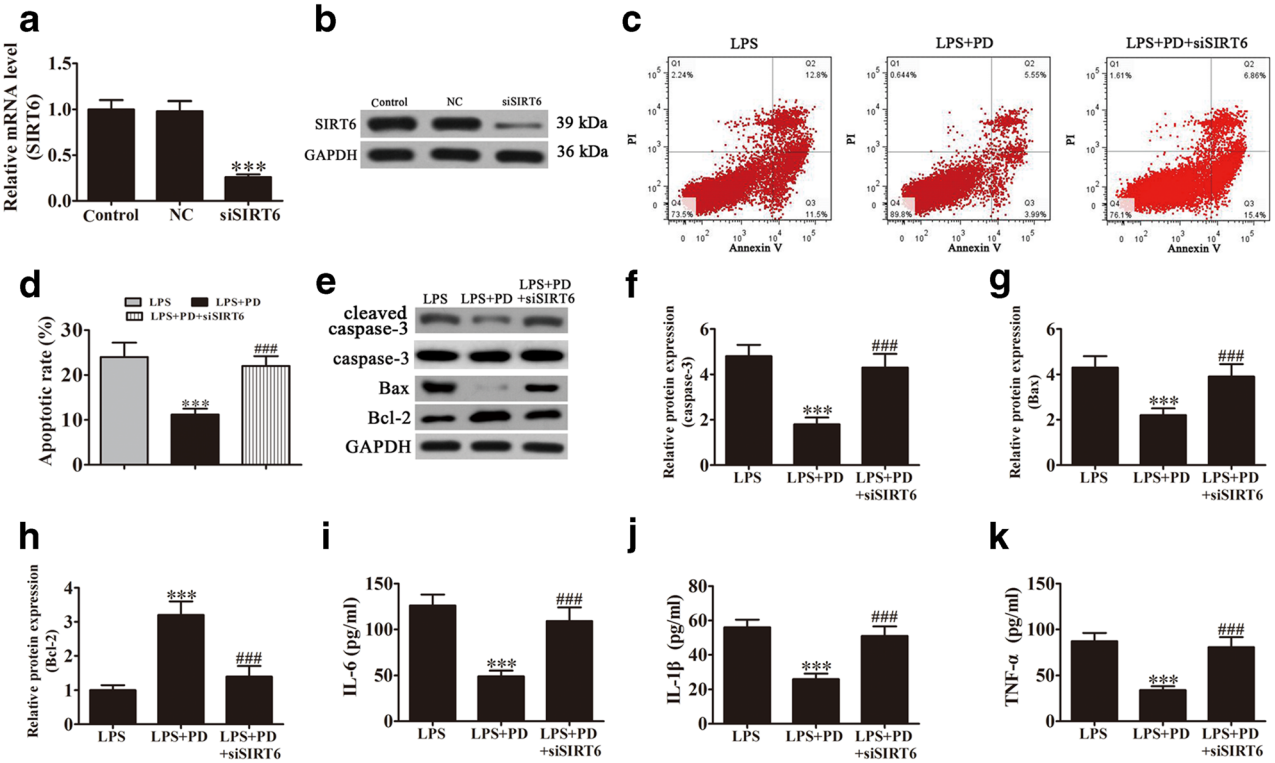

Fig. 2. Downregulation of SIRT6 reversed the suppressive effect of PD on LPS-induced inflammation and apoptosis of H9c2 cells. **a** and **b** RT-PCR and western blotting showed expression of SIRT6 in H9c2 cells after transfection with siRNA against SIRT6 (siSIRT6) or negative control (NC) for 48 h. Data are presented as the mean ± SD. $^{***}P < 0.001$ *versus* controls. **c** Apoptosis was analyzed by flow cytometry after double labeling with AV–FITC and PI. **d** The percentage of apoptotic cells in each group was analyzed. Data are presented as the mean ± SD. $^{***}P < 0.001$ *versus* LPS group. $^{\#\#\#}P < 0.001$ *versus* LPS + PD treatment group. **e**–**h** Western blotting showed expression of caspase-3, Bcl-2, and Bax. GAPDH was used as an endogenous control. Data are presented as the mean ± SD. $^{***}P < 0.001$ *versus* the controls. $^{\#\#\#}P < 0.001$ *versus* LPS treatment group. **i**–**k** Inflammatory factors IL-1β, IL-6, and TNF-α from supernatant were detected by ELISA. Data are presented as the mean ± SD. $^{***}P < 0.001$ *versus* LPS group. $^{\#\#\#}P < 0.001$ *versus* LPS + PD treatment group.

PD Pretreatment Decreased Serum Inflammatory Factor Levels in Rats Subjected to CLP by Promotion of SIRT6 Activation and Myocardial Autophagy

Serum inflammatory factors IL-1β, IL-6, and TNF-α were detected by ELISA after CLP for 48 h. PD pretreatment decreased CLP-induced serum inflammatory factor levels. 3-Ma pretreatment alleviated the inhibitory effect of PD on CLP-induced inflammatory factor expression (Fig. 5). Western blotting showed that expression of SIRT6 was downregulated after CLP. PD pretreatment promoted SIRT6 expression. 3-Ma pretreatment had no effect on PD-induced SIRT6 expression (Fig. 6a, b). It was suggested that SIRT6 had a regulatory effect on autophagy, but suppression of autophagy had no effect on SIRT6 expression. Western blotting confirmed that PD pretreatment promoted Beclin-1 expression and increased the ratio of LC3-II/LC3-I. PD pretreatment decreased P62 expression (Fig. 6c–f).

DISCUSSION

Sepsis-induced myocardial dysfunction (SIMD) is reversible myocardial depression caused by sepsis [28, 29]. Although several studies have reported that endotoxins, inflammatory cytokines, and nitric oxide are related to the pathogenesis of SIMD, the condition is still not completely understood [30]. In this study, we found that autophagy was involved in the progression of SIMD. In the acute stage, suppressed 3-Ma can aggravate the disease by promotion of apoptosis and inflammatory factor expression. This was consistent with previous reports that autophagy activation ameliorates sepsis-induced organ damage [31, 32]. PD pretreatment significantly decreased sepsis-induced myocardial apoptosis and inflammatory factor expression. PD also promotes autophagy activation. Inhibition of autophagy by 3-Ma reversed the protective effect of PD against SIMD.

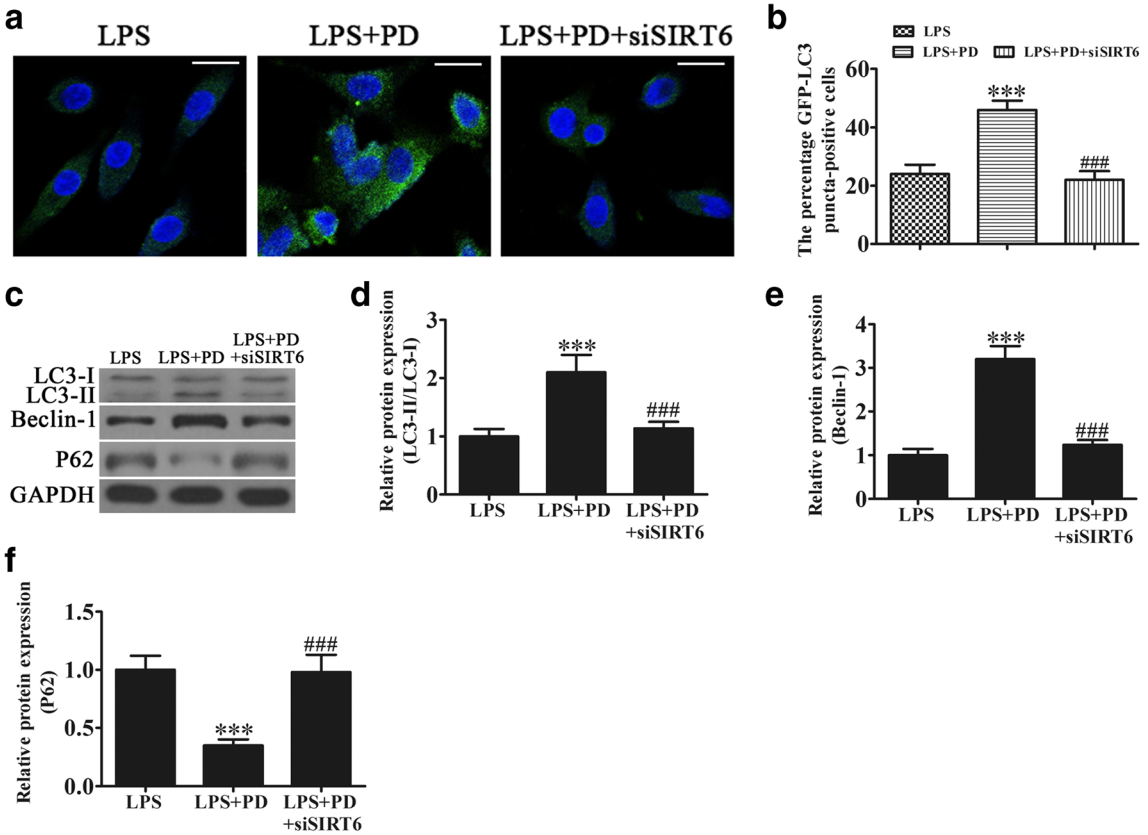

Fig. 3. SIRT6 played an important role in PD-induced autophagy under LPS treatment. **a** and **b** Immunofluorescence showed autophagy puncta in H9c2 cells. Data presented as mean ± SD. Scale bar, 20 μm. ***$P < 0.001$ *versus* LPS group. ###$P < 0.001$ *versus* LPS + PD treatment group. **c**–**f** Western blotting showed expression of P62, LC3, and Beclin-1. Data presented as mean ± SD. ***$P < 0.001$ *versus* LPS group. ###$P < 0.001$ *versus* LPS + PD treatment group.

Table 1. Echocardiographic and Hemodynamic Parameters

	Sham	CLP	CLP + PD	CLP + PD + 3-Ma
Echocardiography				
LVEF (%)	65 ± 3.73	37 ± 3.44***	61 ± 4.34###	38 ± 5.21***
LVFS (%)	36 ± 3.55	19 ± 3.32***	33 ± 2.92###	21 ± 2.36***
LVID(d) (mm)	6.44 ± 0.36	9.25 ± 0.54***	6.15 ± 0.88###	9.03 ± 2.36***
LVID(s) (mm)	3.45 ± 0.62	8.16 ± 0.88***	3.78 ± 0.52###	7.86 ± 0.78***
Hemodynamic				
HR (bpm)	411 ± 25	403 ± 34	434 ± 45	419 ± 35
dP/dt max (mm Hg/s)	5421 ± 341	3216 ± 244***	4963 ± 248###	3021 ± 287***
dP/dt min (mm Hg/s)	4449 ± 248	2608 ± 251***	4215 ± 201###	2821 ± 211***
LVEDP (mmHg)	7.63 ± 0.92	13.44 ± 0.35***	8.04 ± 0.98###	12.41 ± 0.44***

Data are means ± SEM.

LVEF, left ventricular ejection fraction; *LVFS*, left ventricular fraction shortening; *LVID(d)*, left ventricular internal dimension in diastole; *LVID(s)*, left ventricular internal dimension in systole; *HR*, heart rate; *dP/dt max*, maximal slope of systolic pressure increment; *dP/dt min*, minimal slope of diastolic pressure decrement; *LVEDP*, left ventricular end-diastolic pressure

***$P < 0.001$ *versus* the sham group

###$P < 0.001$ *versus* the CLP group

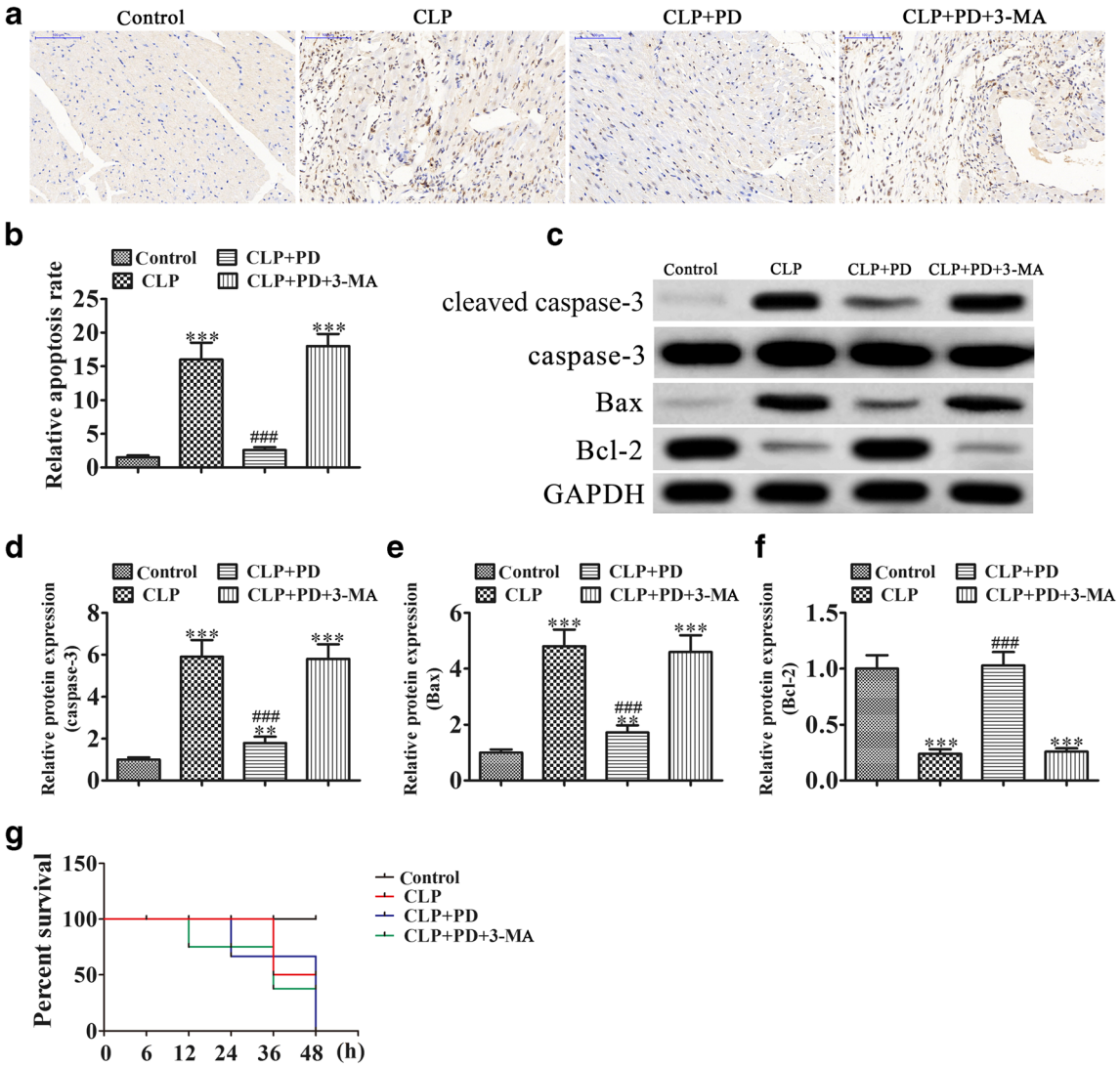

Fig. 4. PD attenuated sepsis-induced myocardial apoptosis and ameliorated survival in rats. **a** and **b** Myocardial apoptosis was measured using TUNEL staining of heart tissue sections. Data are represented as mean ± SD. $^{***}P < 0.001$ *versus* control group. $^{\#\#\#}P < 0.001$ *versus* CLP group. **c**–**f** Western blotting showed expression of caspase-3, Bax, and Bcl-2. GAPDH was used as an endogenous control. Data are presented as mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* control group. $^{\#\#\#}P < 0.001$ *versus* CLP group. **e** The survival of rats was calculated after CLP and 0, 6, 12, 24, 36, and 48 h pretreatment with or without PD (30 mg/kg) or 3-Ma (20 mg/kg).

We found that PD ameliorated SIMD by reducing myocardial apoptosis and inflammatory factor expression. Previous studies have found that PD prevents induction of secondary brain injury after traumatic brain injury by protecting neuronal mitochondria (Li et al., 2019). PD inhibits proliferation and promotes apoptosis of doxorubicin-resistant osteosarcoma through lncRNA TUG1-mediated suppression of Akt signaling [33]. In sepsis, PD inhibited mitochondrial dysfunction in the renal tubular epithelial cells of a rat model of sepsis-induced acute kidney injury [9]. PD also induces septic lung injury in mice *via* upregulation of HO-1 [10, 34]. In this study, we also found that SIRT6 was involved in autophagy regulation after PD treatment. During the past few years, SIRT6 has been investigated extensively, and current evidence shows that it participates in regulation of metabolism, inflammation, and apoptosis [35, 36]. We found that PD pretreatment promoted SIRT6 expression. Silencing SIRT6 significantly suppressed autophagy activation and decreased the protective effect of PD against SIMD. We

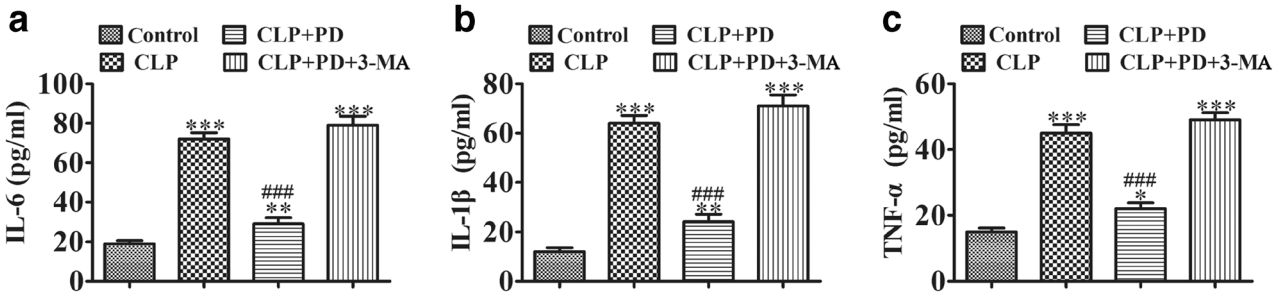

Fig. 5. PD decreased serum inflammatory factor levels in rats subjected to CLP. **a**–**c** Serum inflammatory factors IL-6 (**a**), IL-1β (**b**), and TNF-α (**c**) were detected by ELISA. Data are presented as the mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* control group. $^{\#\#\#}P < 0.001$ *versus* LPS treatment group.

suggest that PD alleviates septic myocardial injury, probably by promotion of SIRT6-mediated autophagy. Previous studies have found that Sirt6 protects podocytes from apoptosis and inflammation by increasing autophagic flux through inhibition of the Notch pathway [37]. Blockage of Sirt6-induced autophagy leads to augmented TREM-1-

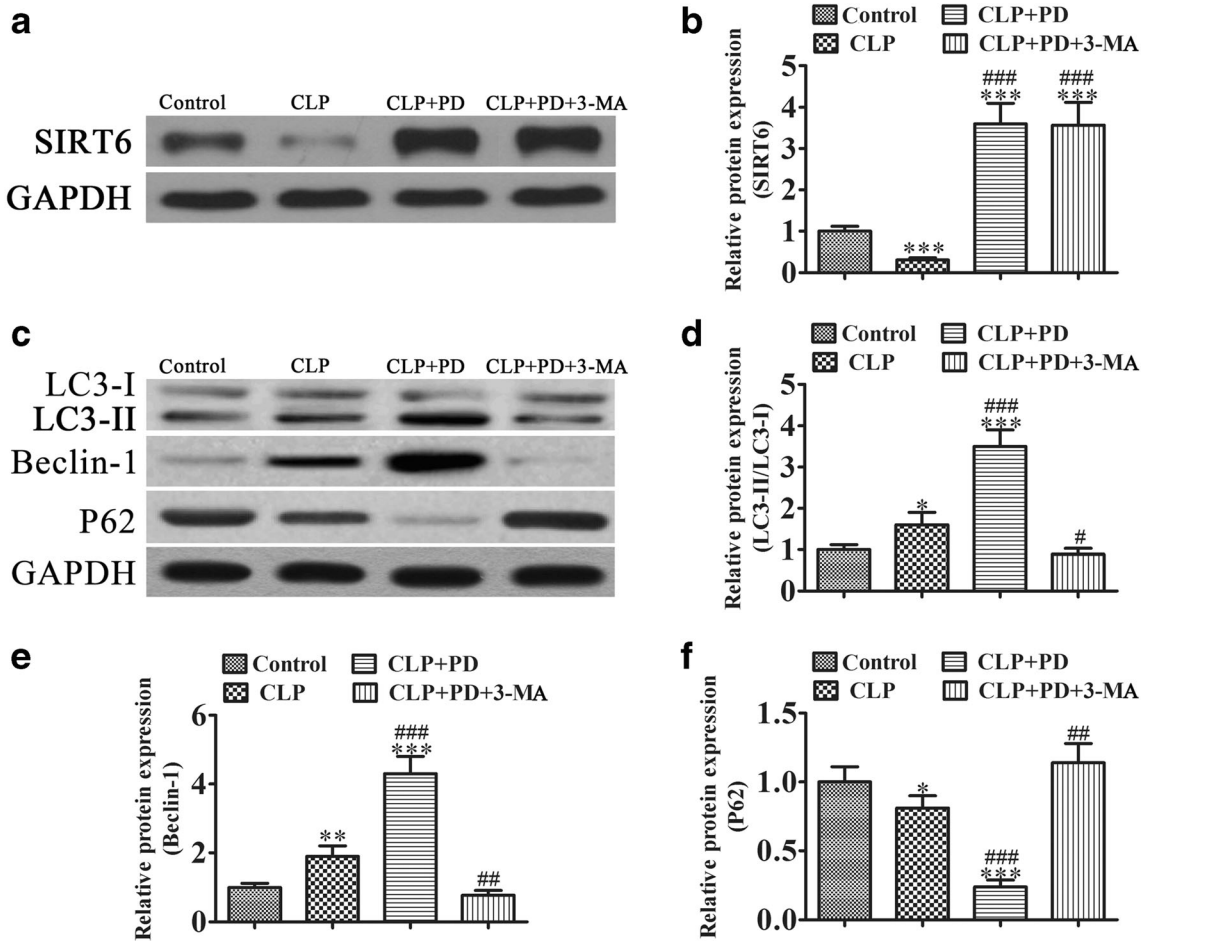

Fig. 6. PD promoted SIRT6 activation and myocardial autophagy. **a** and **b** Western blotting showed expression of SIRT6. GAPDH was used as an endogenous control. Data are presented as the mean ± SD. $^{***}P < 0.001$ *versus* control group. $^{\#\#\#}P < 0.001$ *versus* CLP group. **c**–**f** Western blotting showed expression of P62, LC3, and Beclin-1. GAPDH was used as an endogenous control. Data are presented as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* control group. $^{\#}P < 0.05$, $^{\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *versus* CLP group.

mediated pyroptosis [23]. However, the specific follow-up mechanism for SIRT6 in autophagy needs to be further studied.

In conclusion, our results demonstrate that PD suppresses SIMD *via* inhibition of myocardial apoptosis and inflammatory factor expression probably by promotion of SIRT6-mediated autophagy. PD might be used as a novel treatment for sepsis.

AUTHOR CONTRIBUTIONS

Dongfeng Guo generated and analyzed the data; Xiaoyan Yuan and Guo Chen designed the experiments and drafted the manuscript. All authors approved the version of the manuscript to be published.

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COMPLIANCE WITH ETHICAL STANDARDS

All animal experiments were approved by the Animal Ethics Committee of Pudong New Area Gongli Hospital (Shanghai, China).

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

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