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Brevilin A ameliorates sepsis-induced cardiomyopathy through inhibiting NLRP3 inflammation

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Background: Sepsis is a systemic inflammatory disease, and Brevilin A (BA) has a powerful anti-inflammatory effect. However, whether BA has a similar effect on septic cardiomyopathy remains unclear. This study aimed to investigate the effect and mechanism of BA in septic cardiomyopathy.

Methods: First, a model of septic cardiomyopathy was constructed in vitro and in vivo. The expression of the cardiac injury markers, NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammation factors and its upstream modulator NF-κB was detected by real-time polymerase chain reaction and western blotting. Cardiac function was measured using echocardiography, cell viability was detected using the methyl thiazolyl tetrazolium assay. To further investigate the effects of BA on septic cardiomyopathy, different concentrations of BA were used. The experiment was divided into control group, LPS induced- group, LPS + 2.5, 5.0, 10.0 μM BA treatment group of the vitro model, and the Sham, CLP, CLP + 10, 20, 30 mg/kg BA treatment groups of the rat vivo model. Lastly, cardiac injury, NLRP3 inflammation, and cardiac function were assessed in each group.

Results: The mRNA and protein expression of cardiac inflammation and injury genes were significantly increased in the in vitro and in vivo sepsis cardiomyopathy models. When different concentrations of BA were used in sepsis cardiomyopathy in vivo and in vitro, the above-mentioned myocardial inflammation and injury factors were suppressed to varying degrees, cell viability increased, cardiac function improved, and the survival rate of rats also increased.

Conclusion: BA ameliorated sepsis cardiomyopathy by inhibiting NF-κB/NLRP3 inflammation activation.

Keywords: BA, NF-κB, NLRP3 inflammation, sepsis cardiomyopathy

Introduction

Sepsis is a life-threatening inflammatory disease that is stimulated by bacterial, viral, and fungal infections[1,2]. When sepsis invades the heart, it can cause septic cardiomyopathy and myocardial dysfunction[3]. The characteristics of septic cardiomyopathy include decreased left ventricular ejection fraction (LVEF), low perfusion without ventricular systolic dysfunction and ventricular systolic and/or diastolic dysfunction, resulting in biventricular dilation and cardiac dysfunction[4,5]. Finally, cardiac depression causes death in patients with septic cardiomyopathy. Studies have found that the mortality rate of patients with sepsis cardiomyopathy has increased 2–3 times[6]. Therefore, effective and timely cardioprotective therapies are very important for patients with sepsis.

It has been reported that many mechanisms participate in septic cardiomyopathy, including imbalance of the inflammatory response, apoptosis, mitochondrial dysfunction, and autophagy/mitophagy[7,8]. The NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, composed of NLRP3, apoptosis-associated speck-like protein (ASC), and caspase-1,

HIGHLIGHTS

- Brevilin A (BA) serves as a promising natural medicine for the treatment of septic cardiomyopathy.
- NOD-like receptor family pyrin domain-containing 3 inflammasome and NF-κB activation were increased in septic cardiomyopathy.
- BA inhibited cardiac injury, attenuated the cardiac dysfunction of sepsis cardiomyopathy.
- BA ameliorated sepsis-induced cardiomyopathy by inhibiting NF-κB/NLRP3 inflammation activation, which provides a new approach for the treatment of septic cardiomyopathy.

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exacerbates the production of proinflammatory cytokines[9]. When activated, NLRP3 forms a multiprotein complex that activates caspase-1, which converts procytokines such as ASC, Interleukin-1 beta (IL-1β) to mature forms, thereby promoting inflammation[10,11]. Recent studies have shown that NLRP3 activation plays a critical role in the initiation and progression of the inflammatory response in septic cardiomyopathy[12]. As a significant nuclear transcription factor of the Rel family protein, Nuclear factor-κB (NF-κB) activation is critical for enhancing the expression of the NLRP3 inflammasome[13]. In addition, it mediates various inflammation-related diseases by participating in various stages of inflammation at an early stage[14–16].

As a traditional Chinese medicine, sesquiterpene lactones are a large group of compounds, including Brevilin A (BA), Narjatamolide, Gaillardin. It has been used for several thousands of years and has significant efficacy and relatively minor toxic side effects[17], such as anticancer[18], anti-influenza virus[19], alleviation of liver fibrosis[20], and inhibition of systemic inflammatory response[21]. As a natural sesquiterpene lactone, BA was extracted from the medicinal herb Centipeda minima[22]. BA has a powerful anti-inflammatory effect[23].

Recent research shows that BA is a potent NLRs inflammasome inhibitor, which can significantly affect ASC upstream oligomerization and inhibit NLRP3 inflammasome activation. Importantly, BA significantly attenuated IL-1β secretion and inhibited NLRP3 inflammasome activation in *Escherichia coli*-induced inflammation and MSU-mediated peritonitis mouse models[24]. This study provides a rationale for BA as a small molecule inhibitor for the treatment of inflammation-related diseases induced by NLRP3 inflammasome dysregulation. However, there have been no reports on the effects of BA on cardiomyopathy in sepsis patients. Therefore, this study revealed the effect of BA on septic cardiomyopathy and provided a new therapeutic method for the prevention and treatment of septic cardiomyopathy.

Methods

Animals and reagents

Male Sprague-Dawley rats (6-week-old) weighing 200–25 g was provided by the Experimental Animal Center. The experiments were conducted in accordance with the national standard GB/T35892-2018 'Guidelines for Ethical Review of Laboratory Animal Welfare', the rats were anesthetized by intraperitoneal injection of 2% pentobarbital sodium saline (40 mg/kg; Sigma Chemical Co.). According to the 2020 American Veterinary Medical Association (AVMA) animal euthanasia guidelines released by the AVMA, rats were euthanized for cervical dislocation under deep anesthesia. When the animal experienced respiratory and cardiac arrest, dilated pupils, and the disappearance of the light reflex, after observing for 3 min, the animal died. The humane endpoints in this experiment is based on the evaluation of five aspects of animal status: weight, appearance, measurable clinical signs, and behavior and response to unfounded external stimuli. Severe clinical symptoms are not limited to these five aspects. If the animal has pain or suffering beyond the expectation of the experiment, the impact on animal welfare and the experimental results should be evaluated. BA (MedChemExpress, purity >95%) was purchased from MedChemExpress, LLC. Before the experiment, a 10% fat emulsion (consisting of 0.2% DMSO, 10% PEG300, and 2.5% glycerol) was used to dissolve BA fully by the ultrasonic method, and the final concentrations were 2.5, 5.0, 10.0, 1.8, 3.6, 5.4 mg/ml BA solution.

Experimental grouping

In the in vitro study, the experiment was divided into control, LPS-induced, and 2.5, 5.0, 10.0 μM BA + lipopolysaccharide (LPS)-induced groups. The cells were induced with BA after LPS treatment for 6 h. In the in vivo study, the rats were divided into Sham, cecal ligation and puncture (CLP), 10, 20, and 30 mg/kg BA + CLP-induced groups. There are 8 rats in each group, with a total of 40 rats. In the experiment of calculating rat survival rate, the experiment was divided into Sham (eight rats in the group), CLP-induced (20 rats in the group), and 20 mg/kg BA groups (20 rats in the group), the total number of rats is 48. The mortality rates were 0. In summary, a total of 88 rats were used in the entire experiment. The estimation of sample size is determined by the experience of previously published papers. Rats in each group were injected with BA intraperitoneally or with vehicle (consisting of 10% DMSO, 30% PEG400, and 60% saline) 48 h after CLP surgery. Next, relevant detection and euthanization of rats were conducted after 48 h of BA treatment. During this period, none of the mice experienced any special circumstances set by human endpoints.

The in vivo model of sepsis-induced cardiomyopathy was established by CLP

Because the disease characteristics and progression are similar to those of sepsis in humans, CLP can be used to construct an in vivo model of septic cardiomyopathy[25,26]. Briefly, rats were anesthetized using the above method. It was then fixed on the surgical table in a supine position, and the abdomen was cleaned for skin preparation. A 1 cm incision was made on the lower right side of the anterior abdomen in the cecum area. The cecum was then accurately identified and ligated. After successful cecal ligation, 12 punctures of the cecum were made using a No. 22 needle to induce sepsis. Finally, the cecum was placed back into the abdomen and the peritoneal wall and skin were sutured. For Sham-surgery rats, skin incision and wound suture were performed only under anesthesia, and CLP were not performed. The experimental animals were placed in a controlled environment at 22°C, 50% humidity, and a 12 h dark cycle.

The in vitro model of sepsis-induced cardiomyopathy was established by LPS

Primary neonatal rat cardiac ventricular myocytes (NRCMs) were obtained from neonatal rat (1–3 days). Cardiomyocytes were cultured in Dulbecco's modified Eagle's medium (DMEM) containing fetal bovine serum (10%, FBS, Gibco), penicillin (100 IU/ml), and streptomycin (100 IU/ml). They were then subcultured routinely to a fusion degree of 80% at 37°C under 5% CO2 before further experiments. LPS is the main component of the gram-negative bacterial wall[27] and is mainly composed of three parts: a conservative lipid A segment, a core oligosaccharide segment, and a variable polysaccharide chain 'O antigen', which is an important factor in bacterial-induced sepsis[28]. Previous research found that NRCMs stimulated by 1 μg/m LLPS for 6 h,

an in vitro model of sepsis cardiomyopathy, could be constructed successfully[29,30].

The mRNA expression was detected by real-time polymerase chain reaction

The mRNA expression levels of the myocardial injury factor cardiac troponin (cTnT), creatine kinase-MB (CK-MB), and inflammatory factors NLRP3, ASC, Capase-1 were detected using RT-PCR. First, TRIzol reagent was used for RNA extraction and purification. Specifically, 50–100 mg of tissue was taken, 1 ml of TRIzol was added, and homogenized with a homogenizer. The homogenized sample was placed at 15–30°C for 5 min to completely separate the nucleic acid protein complexes. Then, 0.2 ml of chloroform was then added, and the tube was shaken vigorously for 15 s. The sample was placed at room temperature for 3 min, centrifuged at 10 000 rpm for 10–15 min at 4°C, and divided into three layers: RNA was mainly present in the aqueous phase, and the aqueous layer (about 600 μl, about 60% of the Trizol reagent used) was transferred to a new tube. An equal volume of isopropanol was added to the obtained aqueous solution, mixed well, and placed at – 20°C for 20–30 min. Then RT-PCR was then carried out, and the reaction mixture to 94–98° to activate DNA polymerase. The steps were as follows: denaturation (94–98° for 20–30 s), annealing (50–65° for 20–40 s), and elongation (72°C for 60 s); these steps for 30–35 cycles and extend at 68–74° for 5–10 min. Usually, we set 4–10° as the temperature of the PCR product after the reaction. GAPDH was used as an internal control. The gene expression levels were calculated using the $2-\Delta\Delta Ct$ method. The primer sequences for the genes are as follows, see Table 1.

The protein expression was detected by Western blot analysis

Protein expression was detected by western blot analysis, as described previously[31]. First, appropriate amounts of cells or tissues were collected to obtain protein samples, and the protein concentration was determined using the BCA method. Next, a 10% SDS-PAGE gel was prepared, and an appropriate amount of concentrated SDS-PAGE protein loading buffer was added to the collected protein samples, followed by incubation at 100°C for 3–5 min to fully denature the protein. After cooling to room temperature, the protein sample was directly loaded into the SDS-PAGE gel injection wells. The voltage was usually set at 100V for, 90–120 min. The PVDF membrane was then used for membrane conversion, and the membrane conversion current to 300–400 mA for, 30–60 min. After the membrane transfer was completed, the protein film was immediately placed in a prepared western washing solution and rinse for 1–2 min to remove the membrane transfer solution. Western sealing solution was added again, slowly shaken on a shaker, sealed at room temperature for 60 min, and then probed with primary antibodies against cTnT (1:500, abs146487, Absin). CK-MB (1:500, BYBV-A2087-100, Biovision), NLRP3(1:500, BA3677, BOSTER), ASC (1:500, PA8403H, AtaGenix) and Capase-1(1:500, Biovector122043, Biovector), and NF-κB p65 (1:1000, D14E12, Cell Signaling Technology), overnight at 4°C. Prior to detection, the membranes were incubated with secondary antibodies (rabbit antirat IgG, 1:500, orb27748, Biorbyt) at room temperature for 1 h. Western washing solution was added, and wash for 5–10 min. GAPDH was used as an internal control. Western blot bands were quantified using the Quantity One software. The signals of each band were quantified using the ImageJ software.

Table 1

The primers sequences as follows.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
NLRP3	CTCCAACCATTCTCTGACCAG	ACAGATTGAAGTAAGGCCGG
Capase-1	GCACACGTCTTGCTCTCATT	GCCTCCAGCTCTGTAGTCAT
ASC	GTCACAAACGTTGAGTGGCT	AAGTCCTTGCAGGTCCAGTT
GAPDH	TCATGGATGACCTTGGCCAG	GTCTTCACTACCATGGAGAAGG
NF-κB	AGAGGGGATTTCGATTCCGC	CCTGTGGGTAGGATTTCTTGTTC

The Cardiac function of rats was assessed by M-mode echocardiography

Cardiac function was evaluated using the VividTM E95 echocardiography system (GE Healthcare). In short, the rats were anesthetized according to the above method, fixed in their left lying position on the examination table, the hair was removed from the chest of the rat, and the ultrasound coupling agent was applied. An L8-18i-D PROBE ultrasound probe (GE Healthcare) was used for detection. We then obtained M-mode images and measured the left ventricular diameter (LVID) at systole, left ventricular diameter (LVIDd) at diastole, end diastolic velocity (EDV), and shrink end capacity (ESV), and the LVEF was calculated as (EDV-ESV)/EDV. To ensure the objectivity of the experiment, the above inspections were completed by professional inspectors who were not involved in the study.

Cell viability was analyzed by the Methyl Thiazolyl Tetrazolium (MTT) assay

The specific method was as follows: digest logarithmic growth phase cells with trypsin and prepare a cell suspension, inoculate into a 96 well plate, and incubate in 5% CO2 at 37°C until the cells adhere to the wall and cover the bottom of the well. Treatment was carried out according to the experimental plan, with 4–6 replicates set for each sample concentration. Add 20 μl MTT solution (5 mg/ml, i.e. 0.5% MTT, Sigma Aldrich, St. Louis) was added to each well and incubated for 4 h. Then, add 150 μl DMSO (Dimethyl sulfoxide (DMSO) (Sigma Aldrich, St. Louis) was added to each well. The solution was shaken at low speed (120–140 rpm/min) on a horizontal shaker for 10 min to fully dissolve the crystals. Finally, the 490 nm light absorption value was measured using a microplate reader (Bio-Rad Laboratories Ltd), and cell viability was calculated according to the formula. Proliferation rate (cell viability) = (experimental group blank control)/(negative control blank control) × 100.

Statistical analysis

Data analysis was conducted by professionals who do not understand the grouping of experiments. All values were expressed as the mean ± SD. The Kaplan–Meier method and compared using the log-rank (Mantel-Cox) test were used to compare survival curves. Differences among different groups were compared by one-way analysis of variance (ANOVA) using the GraphPad software. A two-tailed Student's *t*-test was used to compare the two groups $P < 0.05$.

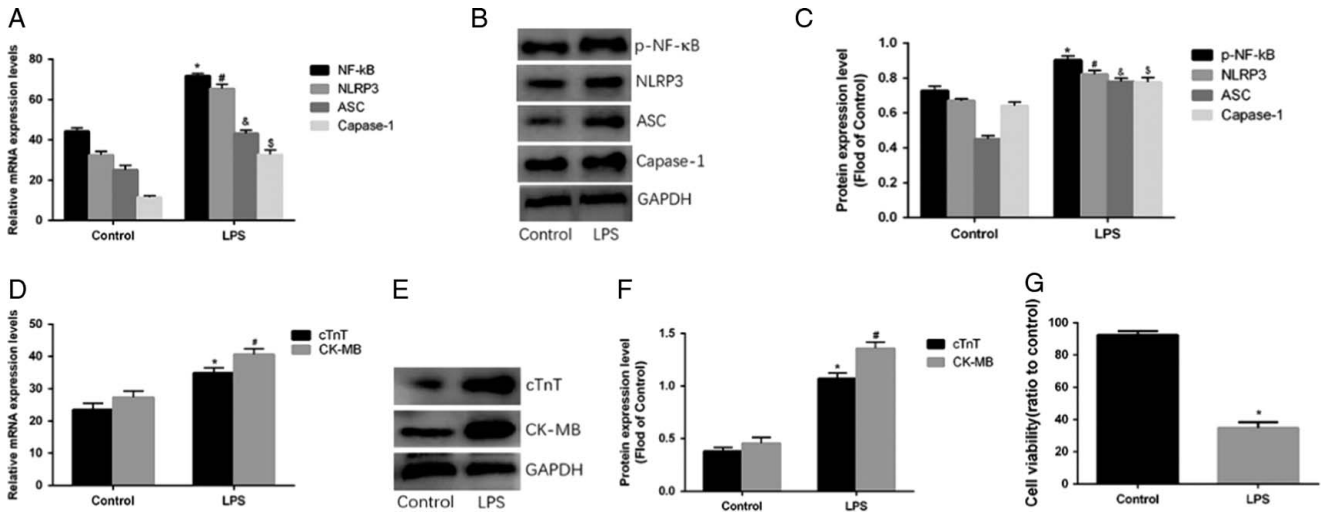

Figure 1. NLRP3 inflammasome and NF-κB activation increased in the vitro model of septic cardiomyopathy. (A) NLRP3, ASC, Capase-1, NF-κB mRNA expression levels. (B) NLRP3, ASC, Capase-1, p-NF-κB protein expression levels. (C) Quantitative analysis NLRP3, ASC, Capase-1, p-NF-κB protein expression levels. (D) cTnT, CK-MB mRNA expression levels. (E) cTnT, CK-MB protein expression levels. (F) Quantitative analysis cTnT, CK-MB protein expression levels. (G) Cell viability in each group. The data are expressed as Mean ± SD. $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\&}P < 0.05$, $^{\$}P < 0.05$ compared with control group ($n = 6$).

Results

NLRP3 inflammasome and NF-κB activation increased in the in vitro model of septic cardiomyopathy

Primary NRCMs were induced by LPS to build an in vitro model of septic cardiomyopathy. First, the cells were divided into control and LPS-induced groups (referred to as the LPS group), and 1 μg/ml LPS was used to induce NRCMs for 6 h in the LPS group. To investigate whether the in vitro model of septic cardiomyopathy was successfully constructed, the myocardial injury marker molecules cTnT and CK-MB were detected by RT-PCR and western blotting. The results showed that the mRNA and protein expression levels of cTnT and CK-MB were significantly higher in the LPS-induced group than in the control group ($n = 6$, $^{*}P < 0.05$, $^{\#}P < 0.05$) (Figs 1D, E, F). In addition, the cell viability in the LPS group was lower than that in the control group (Fig. 1G), and the difference was statistically significant ($n = 6$, $^{*}P < 0.05$). Thus, an in vitro model of septic cardiomyopathy was successfully established.

In order to investigate the inflammatory changes, NLRP3 inflammasome-related molecules NLRP3, ASC, and Capase-1 expression were detected, and NF-κB, a transcription factor that is considered an upstream modulator of the NLRP3 inflammasome, was also detected to assess the activation of the NLRP3 inflammasome and NF-κB. The results showed that the expression levels of NLRP3, ASC, Capase-1, and NF-κB (p65-NF-κB) were significantly higher in LPS-stimulated NRCMs ($n = 6$, $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\&}P < 0.05$, $^{\$}P < 0.05$) (Figs 1A, B, C). The above results show that the NLRP3 inflammasome and NF-κB activation increased in an in vitro model of septic cardiomyopathy.

NLRP3 inflammation and NF-κB were activated in the in vivo model of sepsis cardiomyopathy

First, an in vivo model of sepsis cardiomyopathy was constructed using CLP-induced rats, according to previous methods. The rats in the experimental group were subjected to CLP surgery; 48 h after surgery, myocardial injury, and cardiac function were assessed to evaluate whether the model had been constructed successfully. The results showed that compared with the Sham group, the protein and mRNA expression of cTnT and CK-MB in the CLP treatment group was increased, and the difference was statistically significant (Figs 2 D, E, F) ($n = 6$, $^{*}P < 0.05$, $^{\#}P < 0.05$). In addition, cardiac function was assessed using M-mode echocardiograms the 48 h after the operation. The results showed that LVEF decreased, while LVIDd and LVIDs increased in the CLP-induced group compared with the Sham group, and the difference was statistically significant. (Figs 2G, I, J, K) ($n = 6$, $^{*}P < 0.05$); however, the heart rate changes in the two groups were not statistically significant (Fig. 2H). Therefore, an in vivo model of sepsis-induced cardiomyopathy was successfully constructed by CLP-induced rats.

Next, to understand the inflammatory changes in septic cardiomyopathy rats in vivo, the myocardial NLRP3 inflammation-related molecules NLRP3, ASC, Capase-1, and its upstream modulator NF-κB were detected. The results showed that compared with the Sham group, the protein and mRNA expression of NLRP3, ASC, Capase-1, and NF-κB increased in the CLP treatment group, and the difference was statistically significant (Figs 2 A, B, C) ($n = 6$, $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\&}P < 0.05$, $^{\$}P < 0.05$). Thus, NLRP3 inflammation and NF-κB are activated in an in vivo model of septic cardiomyopathy.

BA inhibited cardiomyocyte injury and increased cell viability in the in vitro model of sepsis cardiomyopathy

To study the effect of BA on cardiomyocyte injury and viability in an in vitro model of septic cardiomyopathy, the experiment was divided into the control group, LPS-induced group, LPS + 2.5 μM BA treatment group, LPS + 5.0 μM BA treatment group, and LPS + 10.0 μM BA treatment group. After 6 h, the cardiomyocyte injury marker molecules cTnT and CK-MB were detected using qPCR and western blotting, and cell viability was tested using the

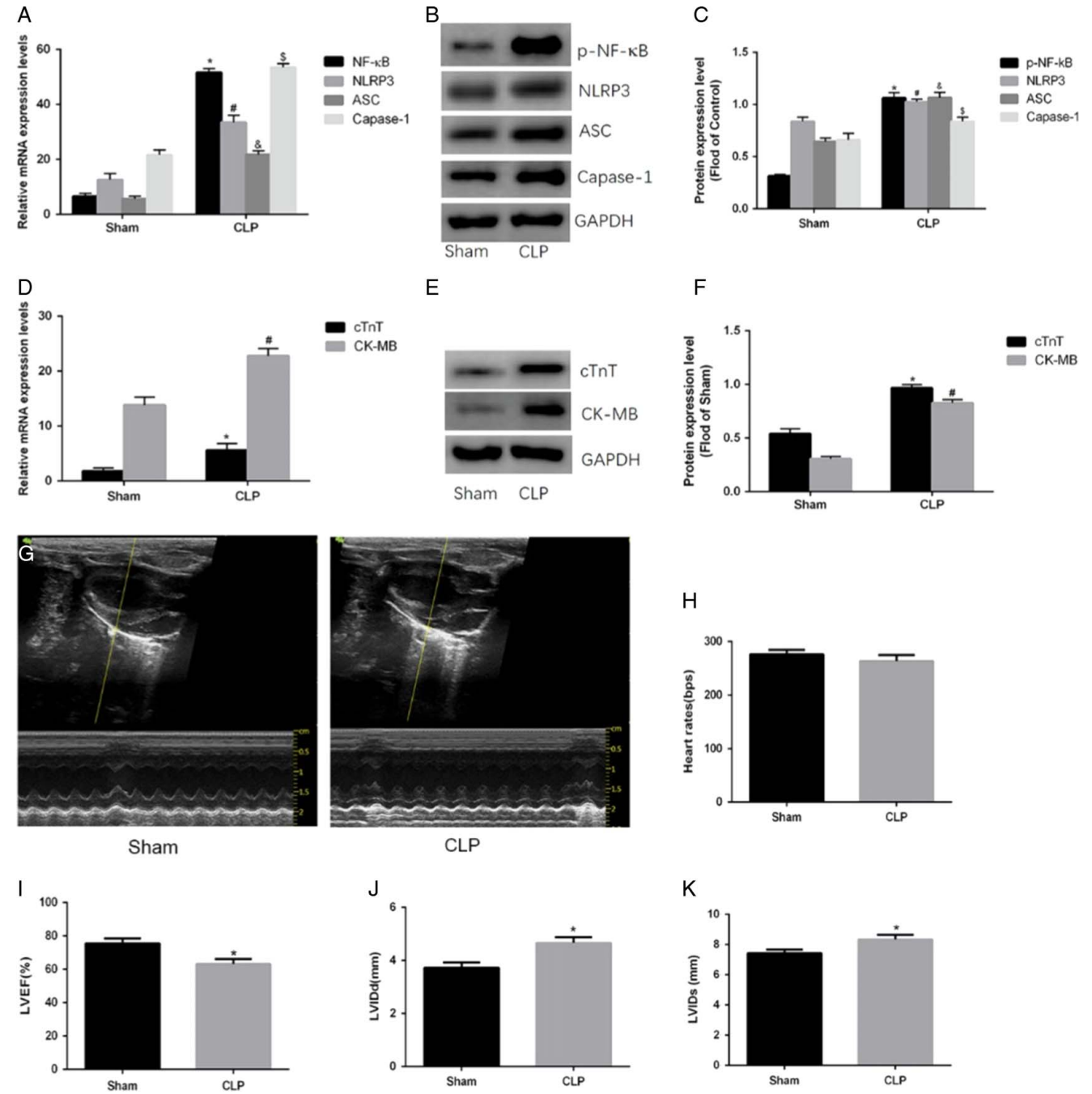

Figure 2. NLRP3 inflammation and NF-κB were activated in the vivo model of sepsis cardiomyopathy. (A) NLRP3, ASC, Capase-1, NF-κB mRNA expression levels in vivo. (B) NLRP3, ASC, Capase-1, p-NF-κB protein expression levels in vivo. (C) Quantitative analysis NLRP3, ASC, Capase-1, p-NF-κB protein expression levels in vivo. (D) cTnT, CK-MB mRNA expression levels. (E) cTnT, CK-MB protein expression levels. (F) Quantitative analysis cTnT, CK-MB protein expression levels. The data are expressed as Mean ± SD. (G) Representative M-mode echocardiograms obtained from rats in each group. (H) Heart rates (bps). (I) LVEF (%); (J) LVIDd (mm); (K) LVIDs (mm). The data are expressed as Mean ± SD. Left ventricle internal diameter at systole (LVIDs), Left ventricle internal diameter at diastole (LVIDd), Left ventricle ejection fraction (LVEF). ($n = 6$, $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\&}P < 0.05$, $^{\$}P < 0.05$).

Methyl Thiazolyl Tetrazolium (MTT) assay. The results showed that the mRNA expression levels of *cTnT, CK-MB* decreased in the 5.0 and 10.0 μM BA treatment groups compared to the LPS group, and the difference was statistically significant ($n = 6$, $^{*}P < 0.05$, $^{\#}P < 0.05$) (Figs 3A, B). The protein expression levels of cTnT and CK-MB were decreased in the BA treatment group at different concentrations (2.5, 5.0, and 10.0 μM), and the difference was statistically significant ($n = 6$, $^{*}P < 0.05$, $^{\#}P < 0.05$) (Figs 1C, D, and E). It should be noted that there was no statistically significant change in *cTnT, CK-MB* mRNA expression when cardiomyocytes were treated with 2.5 μM BA.

Then, the results showed that compared with the LPS induced-group, the cell viability were increased in 5.0 μM and 10.0 μM BA treatment groups, and the difference was statistically significant

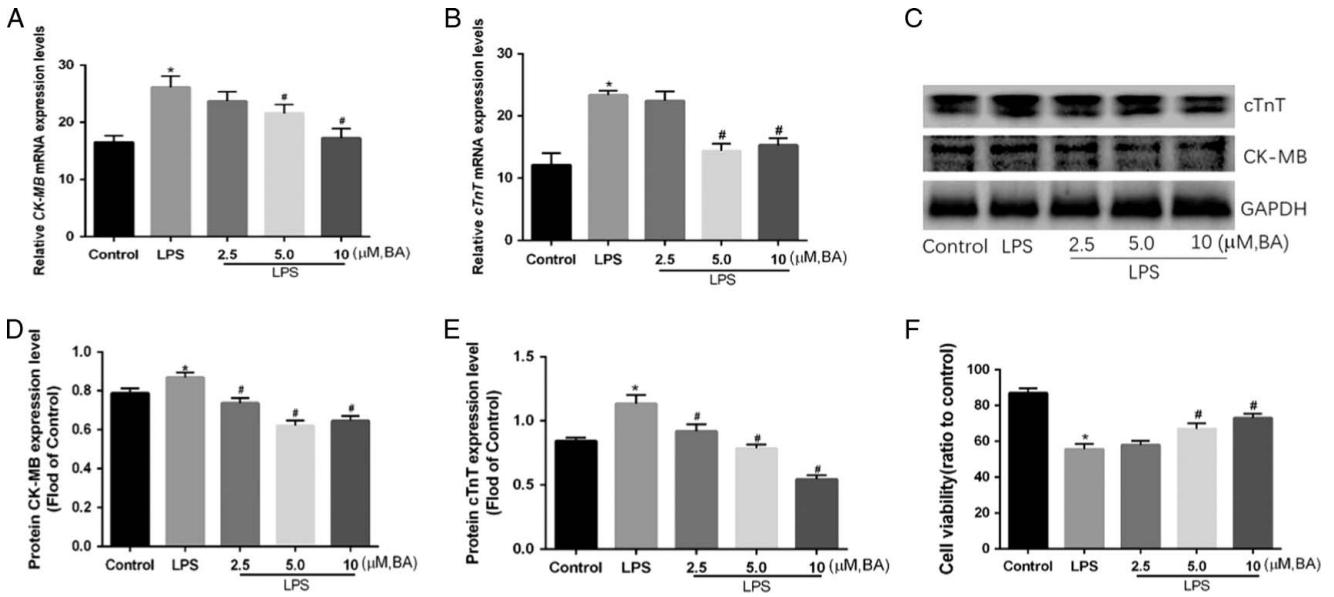

Figure 3. BA inhibited cardiomyocyte injury and increased cell viability in the vitro model of sepsis cardiomyopathy. (A) CK-MB mRNA expression levels. (B) cTnT mRNA expression levels. (C) cTnT, CK-MB protein expression levels. (D) Quantitative analysis CK-MB protein expression levels. (E) Quantitative analysis cTnT protein expression levels. (F) Cell viability in each group; The data are expressed as Mean ± SD. $^{*}P < 0.05$ compared with control group, $^{\#}P < 0.05$ compared with LPS-induced group ($n = 6$).

($n = 6$, $^{*}P < 0.05$, $^{\#}P < 0.05$) (Fig. 3F). However, although the viability of the 2.5 μM BA treatment group was increased also, the difference was not statistically significant. Thus, BA inhibited cardiomyocyte injury and increased cell viability in an in vitro model of sepsis cardiomyopathy, and only when the drug concentration reaches 5.0 μM, BA can exert the best therapeutic effect.

BA alleviated NF-κB and NLRP3 inflammation activation in the in vitro model of sepsis cardiomyopathy

To study the effect of BA on NLRP3 inflammation in an in vitro model of sepsis cardiomyopathy, the protein and mRNA expression of NLRP3 inflammation marker molecules NLRP3, ASC, Capase-1, and its upstream modulator NF-κB were detected. The results showed that compared with the LPS group, the protein and mRNA expression of NLRP3, ASC, Capase-1, and NF-κB decreased to varying degrees, and the difference was statistically significant (Fig. 4) ($n = 6$, $^{\#}P < 0.05$). Specifically, there was no statistically significant change in the mRNA expression of NLRP3, ASC, Capase-1, NF-κB, or protein expression of p-NF-κB and ASC when treated with 2.5 μM BA. Thus, BA treatment alleviated NF-κB and NLRP3 inflammation activation in the in vitro model of sepsis cardiomyopathy only when BA concentration reaches 5.0 μM.

BA attenuated cardiac dysfunction in the rat vivo model of sepsis cardiomyopathy

To further determine the effect of BA on cardiac function in the in vivo model of sepsis cardiomyopathy, rats were injected with different concentrations of BA (10, 20, and 30 mg/kg) for consecutive days after the CLP operation and were divided into Sham, CLP, CLP + 10, 20, and 30 mg/kg BA groups (eight rats in each group). LVEF, LVIDd, and LVIDs were measured to estimate the cardiac function of rats the 48 h after the operation. The results showed that compared with the Sham group, the LVEF decreased and the LVIDd and LVIDs increased in the CLP group, and the difference was statistically significant ($n = 6$, $^{*}P < 0.05$) (Fig. 5). However, the 48 h after treatment with different concentrations of BA, the LVEF increased, and the LVIDd and LVIDs decreased to varying degrees in the 10, 20, and 30 mg/kg BA groups compared with the CLP group, and the differences were statistically significant. ($n = 6$, $^{\#}P < 0.05$) (Fig. 5).

Among them, 10 mg/kg BA was not sufficient to improve the LVEF, LVIDd, and LVIDs of rats, and the difference was not statistically significant. The results indicated that the heart rate changes in each group were not statistically significant. In summary, BA attenuates cardiac dysfunction in a rat model of septic cardiomyopathy.

BA inhibited NF-κB and NLRP3 inflammasome activation in the rat vivo model of sepsis cardiomyopathy

The experimental groups, as described above, and the protein and mRNA expression of the NLRP3 inflammation marker molecules NLRP3, ASC, Capase-1, and its upstream modulator NF-κB were detected the 48 h after the operation. The results showed that NLRP3, ASC, Capase-1, NF-κB decreased to varying degrees in the 10, 20, and 30 mg/kg BA groups compared with the CLP group, and the difference was statistically significant ($n = 6$, $^{\#}P < 0.05$) (Fig. 6). However, the effect of 10 mg/kg BA on p-NF-κB protein expression was not statistically significant. In summary, BA inhibits NF-κB and NLRP3 inflammasome activation in a rat model of septic cardiomyopathy.

BA prevented cardiac injury and improved the survival of septic cardiomyopathy rats

To understand the effect of BA on myocardial injury in septic cardiomyopathy rats, the protein and mRNA expression of

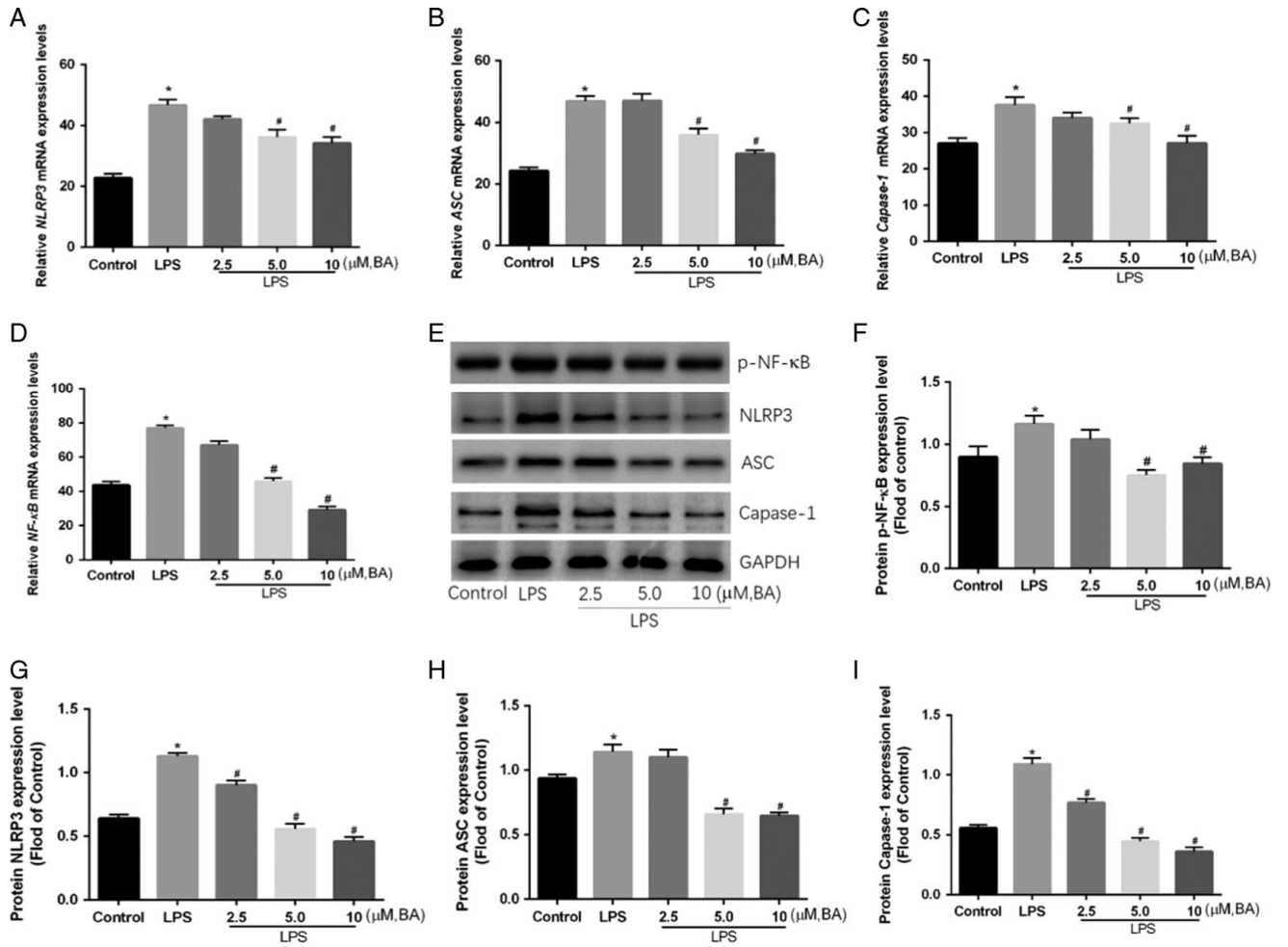

Figure 4. BA alleviated NF-κB and NLRP3 inflammation activation in the vitro model of sepsis Cardiomyopathy. (A) NLRP3 mRNA expression levels. (B) ASC mRNA expression levels. (C) Capase-1 mRNA expression levels. (D) NF-κB mRNA expression levels. (E) p-NF-κB, NLRP3, ASC, Capase-1 protein expression levels. (F) Quantitative analysis p-NF-κB protein expression levels. (G) Quantitative analysis NLRP3 protein expression levels. (H) Quantitative analysis ASC protein expression levels. (I) Quantitative analysis Capase-1 protein expression levels. The data are expressed as Mean ± SD. $^{*}P < 0.05$ compared with control; $^{\#}P < 0.05$ compared with LPS group ($n = 6$).

myocardial injury marker molecules cTnT and CK-MB were detected by qPCR and western blotting on the 48 h after the operation. The results showed that cTnT and CK-MB levels decreased to varying degrees in the 10, 20, and 30 mg/kg BA groups compared with those in the CLP group, and the difference was statistically
significant ($n = 6$, $^{\#}P < 0.05$) (Figs 7A–E). Thus, BA prevents myocardial injury in rats with septic cardiomyopathy.

To study the effect of BA on the survival rate of septic cardiomyopathy rats, the experiment was divided into Sham (8 rats in the group), CLP-induced (20 rats in the group), and 20 mg/kg BA groups (20 rats in the group), and the survival of rats in each group was observed within 6 days after surgery (Table 2 and Fig. 7F). The results showed that the number of deaths of rats in the Sham group was 0 in the past 6 days, that is, the survival rate was 100%. Compared with the Sham group, the overall survival rate of the CLP group was reduced by only 4.761905%, and the difference was statistically significant ($P = 0.0023$). The overall survival rate of the 20 mg/kg BA group increased by 43.315%, and the difference was statistically significant ($P = 0.0465$). After autopsy, the causes of death were determined to be heart failure and explosive sepsis. This showed that BA increased the survival rate of rats with septic cardiomyopathy.

Discussion

In this study, an in vitro model of septic cardiomyopathy was constructed using LPS. To clarify the changes in cardiac inflammatory response and injury in the process, the expression of myocardial injury marker molecules-cTnT, CK-MB, and NLRP3 inflammation marker molecules NLRP3, ASC, Capase-1, and its upstream modulator NF-κB were estimated by RT-PCR and Western blotting. The results showed that NLRP3 inflammation and its upstream modulator, NF-κB, were activated in an in vitro model of septic cardiomyopathy. Simultaneously, the myocardial injury was aggravated. Surprisingly, we obtained similar experimental conclusions for in vivo models. This indicates that during septic

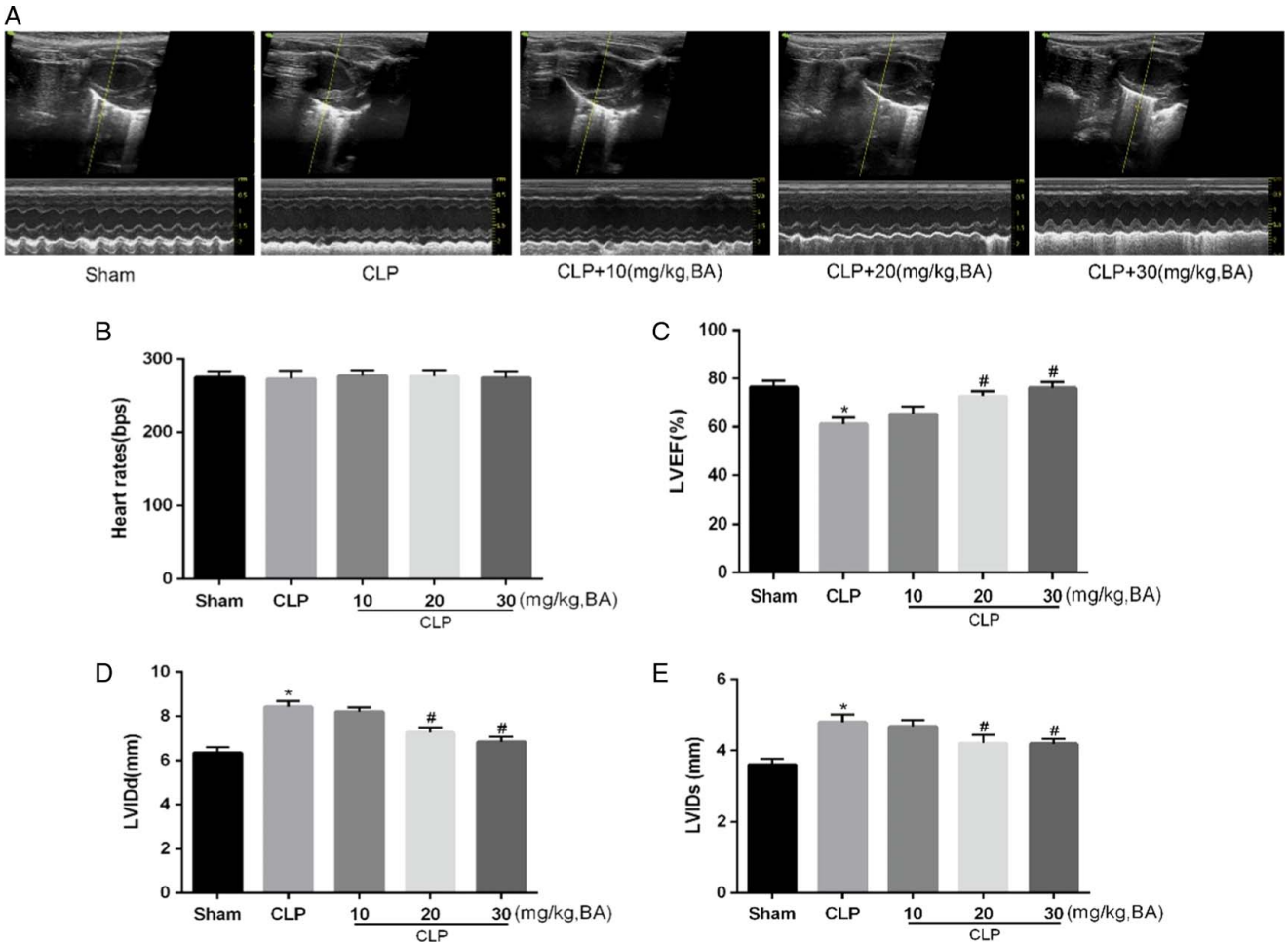

Figure 5. BA treatment attenuated cardiac dysfunction in the vivo model of sepsis cardiomyopathy. (A) Representative M-mode Echocardiograms in each group. (B) Heart rates (bps). (C) LVEF (%). (D) LVIDd (mm). (E) LVIDs (mm). The data are expressed as Mean ± SD. $^{*}P < 0.05$ compared with Sham group; $^{\#}P < 0.05$ compared with CLP group ($n = 6$). CLP, cecal ligation and puncture.

cardiomyopathy, the heart exhibited significant NLRP3 inflammatory activation and further exacerbated myocardial damage.

Currently, the evaluation of cardiac function in patients with sepsis cardiomyopathy is mainly based on echocardiogram indexes such as LVEF, left ventricular end-diastolic dimension (LVEDD), left ventricular end diastolic volume (LVEDV), left ventricular internal dimension diastole (LVIDD), left ventricular internal dimension systole (LVIDS), etc. When LVEF smaller than or equal to 0.50, sepsis cardiomyopathy can be diagnosed[32]. Moreover, N-terminal brain natriuretic peptide (NT-pro BNP) is also a commonly used and important indicator for judging cardiac dysfunction, which is mainly synthesized and secreted by ventricular myocytes. The level of NT-pro BNP provides an early diagnosis of cardiac dysfunction in patients with sepsis cardiomyopathy[33].

Next, in order to further explore the changes in cardiac function in rats with sepsis cardiomyopathy, we established an in vivo model of CLP-induced sepsis cardiomyopathy by CLP induced rats, according to previous methods. The cardiac function of rats was detected by M-mode echocardiograms the 48 h after the operation, and the results showed that the LVEF decreased, while LVIDd and LVIDs increased in septic cardiomyopathy rats compared to healthy group rats. This indicates that rats with sepsis-induced cardiomyopathy have decreased cardiac function. In 1921, septic cardiomyopathy was widely defined as an acute cardiac dysfunction unrelated to myocardial ischemia. Its clinical manifestations include arrhythmia, left or right ventricle injury during systolic or diastolic, with or without cardiac output reduction[34,35]. Previous studies have shown that patients with sepsis and elevated serum troponin (cTn) levels are more likely to have left ventricular dysfunction and significantly higher mortality[34]. Unlike heart diseases, such as heart failure, valvular heart disease, myocardial infarction, and myocardial hypertrophy, the main changes in cardiac function in septic cardiomyopathy are well resuscitated without myocardial dysfunction or fluid responsiveness, hyperkinetic profile, LV systolic dysfunction, RV failure, and persistent hypovolemia[36]. Fortunately, our results were consistent with those of previous studies.

As a sesquiterpene lactone, BA was isolated from the medicinal herb Centipeda minima. Previous studies have shown that BA inhibits various inflammatory reactions. In LPS-induced acute lung injury in vivo, BA inhibited IKKα/β-mediated activation of NF-κB signaling, thereby reducing inflammation activation

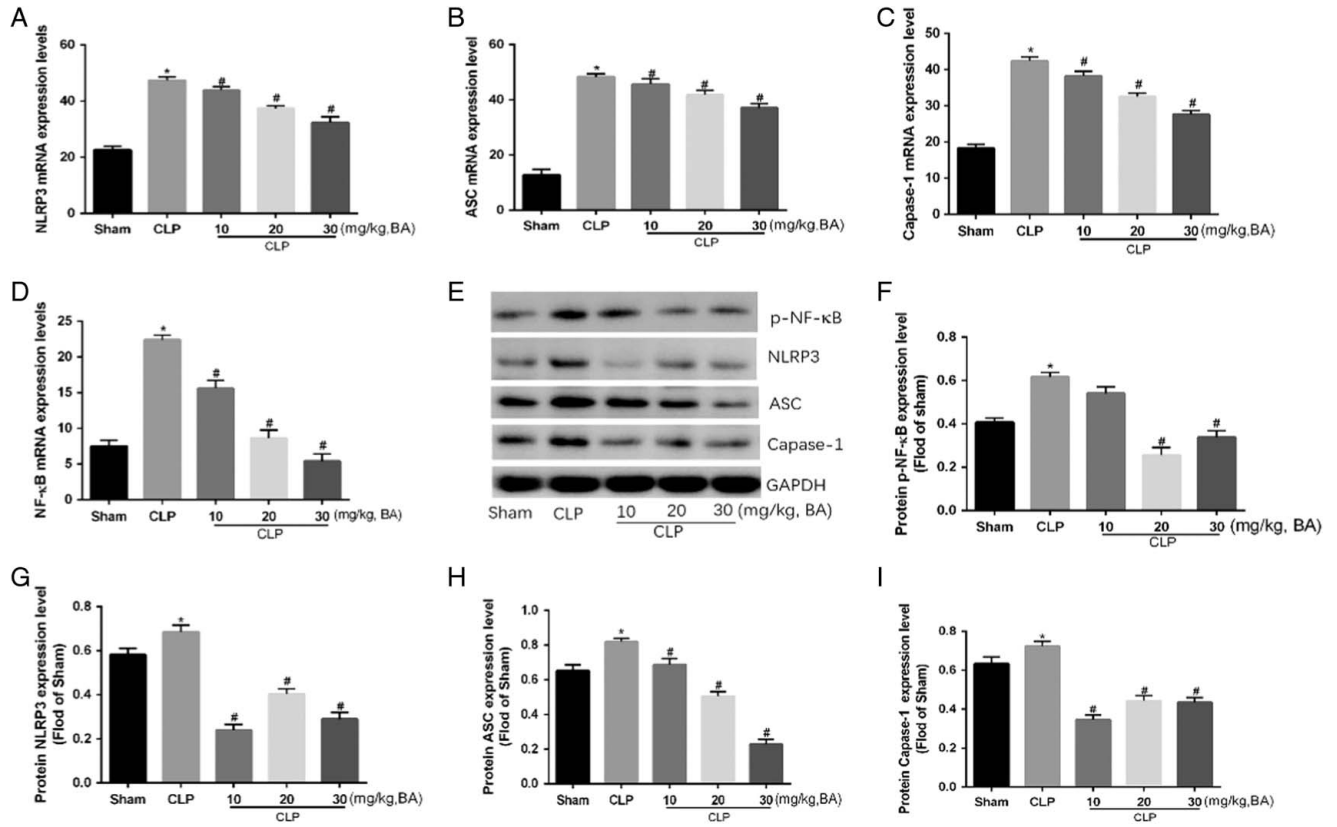

Figure 6. BA inhibited NF-κB and NLRP3 inflammasome activation in the rat vivo model of sepsis cardiomyopathy. (A) NLRP3 mRNA expression levels. (B) ASC mRNA expression levels. (C) Capase-1 mRNA expression levels. (D) NF-κB mRNA expression levels. (E) p-NF-κB, NLRP3, ASC, Capase-1 protein expression levels. (F) Quantitative analysis p-NF-κB protein expression levels. (G) Quantitative analysis NLRP3 protein expression levels. (H) Quantitative analysis ASC protein expression levels. (I) Quantitative analysis Capase-1 protein expression levels. The data are expressed as Mean ± SD. $^{*}P < 0.05$ compared with Sham; $^{\#}P < 0.05$ compared with CLP group ($n = 6$). CLP, cecal ligation and puncture.

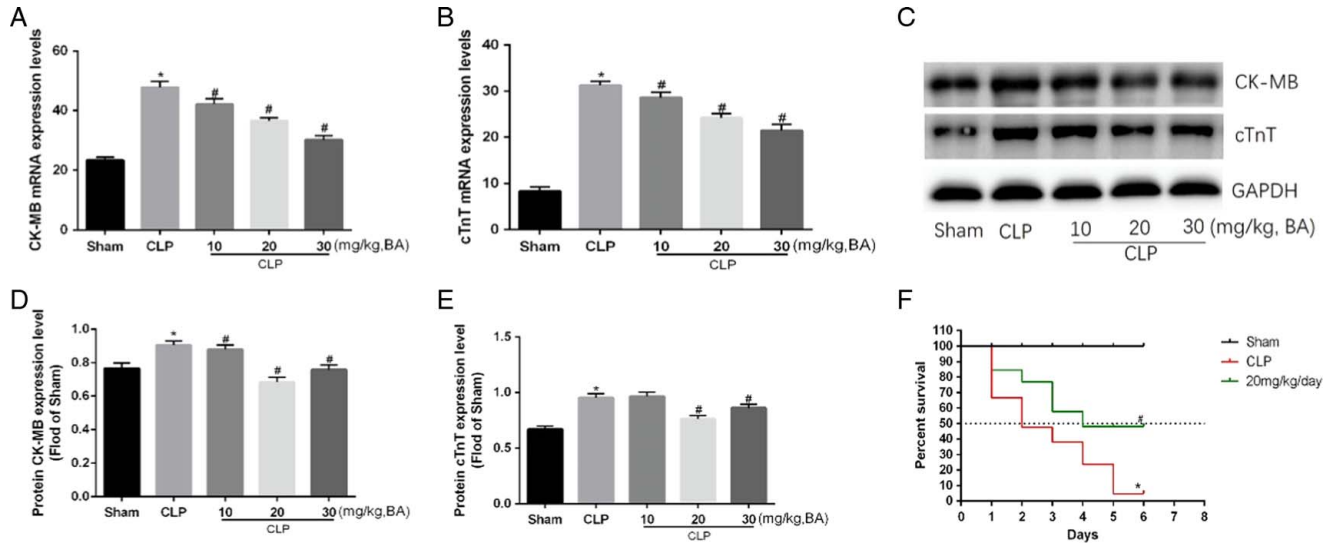

Figure 7. BA prevented myocardial injury and improved survival of septic cardiomyopathy rats. (A) CK-MB mRNA expression levels. (B) cTnT mRNA expression levels. (C) cTnT, CK-MB protein expression levels. (D) Quantitative analysis CK-MB protein expression levels. (E) Quantitative analysis cTnT protein expression levels. The data are expressed as Mean ± SD. $^{*}P < 0.05$ compared with Sham; $^{\#}P < 0.05$ compared with CLP group ($n = 6$). CLP, cecal ligation and puncture. (F) the survival rate of rats in each group. The red line represents the survival of CLP group rats ($n = 20$) decreased compared with Sham group ($n = 8$). (4.76 vs. 100%), $^{*}P < 0.05$; The green line represents the survival of rats treated with 20 mg/kg BA ($n = 20$) was improved compared with the CLP group ($n = 20$) within 6 days postsurgery (48.078 vs. 4.762%, respectively) ($^{\#}P < 0.05$). Sham rats were used as control. The data are expressed as the Mean ± SD. CLP, cecal ligation and puncture.

Table 2

Survival rate of rats in each group every day within 6 days postsurgery.

Days	Sham	CLP	20 mg/kg/day
0.00	100.00	100.00	100.00
1.00	100.00	66.67	84.62
2.00	100.00	47.62	76.92
3.00	100.00	38.10	57.69
4.00	100.00	23.81	48.08
5.00	100.00	4.76	48.08
6.00	100.00	4.76	48.08

in vitro[24]. However, there is still no relevant research on the effects of BA on sepsis-induced cardiomyopathy. To investigate the therapeutic effect and related mechanisms of BA in septic cardiomyopathy, we used different concentrations of BA to treat cells or by intraperitoneal injection into septic rats. The results showed that BA inhibited cardiomyocyte injury, alleviated NF-κB and NLRP3 inflammation activation, and increased cell viability in an in vitro model of sepsis cardiomyopathy. BA exerted the best therapeutic effect only when the drug concentration reaches 5.0 μM, BA can exert the best therapeutic effect. More importantly, in a rat model of sepsis cardiomyopathy, BA inhibited NF-κB and NLRP3 inflammatory activation to prevent myocardial injury and cardiac dysfunction. Previous studies have indicated that BA suppresses NLRP3 inflammasome activation by interfering with upstream ASC oligomerization. Experiments have also shown that BA markedly reduced the secretion of IL-1β and suppressed the NLRP3 inflammasome in a peritonitis model[27]. These findings are consistent with our conclusions.

To further determine the effect of BA on cardiac function in the in vivo model of sepsis cardiomyopathy, rats were injected intraperitoneally with different concentrations of BA (10, 20, 30 mg/kg) for consecutive days after CLP operation. At the 48 h after treatment with different concentrations of BA, the results showed that the LVEF increased, and the LVIDd and LVIDs decreased to varying degrees in the different concentrations of BA-induced group compared with the untreated CLP group. The results indicated that the heart rate changes in each group were not statistically significant. In summary, BA attenuates cardiac dysfunction in a rat model of septic cardiomyopathy. Similarly, BA treatment increased the survival rate of rats with septic cardiomyopathy. Previously, Yang *et al.*[37] proved that inhibition of the NLPR3 inflammasome in the heart mediates LPS-induced cardiac dysfunction, inflammation, and apoptosis to exert cardioprotective effects. In addition, Busch *et al.*[12] thought that NLRP3-mediated IL-1β activation played a critical role in septic cardiomyopathy and improved cardiac dysfunction. Surprisingly, their results were consistent with those of the present study.

Therefore, this study indicated that BA alleviated NF-κB and NLRP3 inflammation activation and inhibited cardiomyocyte injury in in vitro and in vivo models of septic cardiomyopathy. In addition, BA increased cell viability in an in vitro model of septic cardiomyopathy, attenuated cardiac dysfunction in a rat model of septic cardiomyopathy, and improved the survival of septic cardiomyopathy rats. This finding is consistent with the expected results.

However, owing to the limitations of the experimental conditions and time, there are still many shortcomings in this study. We did not study the effects of BA on myocardial autophagy, apoptosis, oxidative stress, mitochondrial function, energy metabolism, etc. of septic cardiomyopathy, or on related signaling pathways, such as AMPK/ERK and PI3K/AKT. Although different concentrations of BA have been used in experimental research, we failed to explore the optimal concentration of BA and the possible toxic effects when it was applied. However, this will be the focus of our future research.

Conclusion

BA may serve as a promising natural medicine for the treatment of septic cardiomyopathy. This mechanism may be related to the inhibition of NF-κB the NLRP3 inflammasome activation.

Ethical approval

Ethical approval for this study (No. is 20220701-1.) was provided by the Ethical Committee No.1. Clinical College of Wuhan University, Wuhan, China on 1 July 2022.

Consent

The experiments were conducted in accordance with the national standard GB/T35892-2018 'Guidelines for Ethical Review of Laboratory Animal Welfare'. Details of any images can be published, and authors providing consent have been shown the article contents to be published.

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Author contribution

Y.F.L.: wrote and revised the manuscript; W.Q.L.: conducted RT-PCR experiments; N.D.H.: conducted Western blotting experiments; B.A.: conducted cell culture and cell viability testing; H.X.X.: performed cardiac echocardiography detection and related data statistics; X.G.: analyzed all experimental data; H.X. and Z.C.: designed experiments. All the authors have read and approved the final version of the manuscript.

Conflicts of interest disclosure

The authors declare that they have NO affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

Research registration unique identifying number (UIN)

The study has no human studies.

Guarantor

Hao xia.

Data availability statement

All relevant data are within the paper and its Supporting Information files.

Provenance and peer review

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