Effect of dobutamine on intrinsic myocardial function and myocardial apoptosis in septic rats with myocardial dysfunction

Xiang-Xu Tang
School of Medicine, Jinan University
Ya-Qian Xu
School of Medicine, Jinan University
Xiao-Meng Dai
School of Medicine, Jinan University
Yun Xing
School of Medicine, Jinan University
Duo-Meng Yang
School of Medicine, Jinan University
Qing-Yang Huang
School of Medicine, Jinan University
Hong-Mei Li
School of Medicine, Jinan University
Xiu-Xiu Lv
School of Medicine, Jinan University
Yi-Yang Wang
School of Medicine, Jinan University
Da-Xiang Lu
School of Medicine, Jinan University
Hua-Dong Wang (owanghd@jnu.edu.cn)

Research

Keywords: Sepsis, Intrinsic myocardial dysfunction, Dobutamine, Cardiomyocyte apoptosis

DOI: https://doi.org/10.21203/rs.3.rs-51092/v1

Abstract

Background

Dobutamine (DOB) has been recommended as the first-line inotrope for septic patients with low cardiac output, but its long-term impact on intrinsic myocardial dysfunction during sepsis remains unclear. This study investigated the long-term effect of DOB on intrinsic myocardial function and cardiomyocyte apoptosis in sepsis.

Methods

Male Sprague-Dawley rats were randomly divided into sham and cecal ligation and puncture (CLP) groups. The intrinsic myocardial function and other organ functions were measured at different time points, the inflammatory response and serum biomarkers of myocardial injury were also determined. In separate experiments, the effect of DOB (5 or 10 μg/kg) treatment on survival, intrinsic myocardial function, serum and myocardial cytokines and myocardial apoptosis were measured in septic rats.

Results

The mortality rate of septic rats was 70% on day 10 after CLP. At 6 h after CLP, the left ventricular ± dP/dt were significantly depressed, serum tumor necrosis factor (TNF) −α level, cardiac TNF-α, intercellular adhesion molecule and vascular cell adhesion molecule-1 (VCAM-1) mRNA, and VCAM-1 protein levels were increased, but not serum cTnI, N-terminal pro-brain natriuretic peptide (NT-proBNP), heart-type fatty acid-binding protein (H-FABP), creatinine and urea nitrogen concentrations as well as lung wet-dry weight ratios in CLP group compared with those in sham group. At 9 h after CLP, serum alanine aminotransferase and aspartate aminotransferase activities were higher in CLP rats than controls. At 6 h after CLP, treatment with DOB did not affect the left ventricular ± dP/dt, the levels of TNF-α, interleukin (IL) − 1β and IL-6 in the serum and myocardium as well as cardiomyocyte apoptosis at 20 h after CLP. However, administration of 10.0 μg/kg DOB at 6 h after CLP significantly increased serum IL-10 level and improved survival in septic rats.

Conclusions

The intrinsic myocardial depression occurs earlier than hepatic and renal dysfunction in severe sepsis and serum cTnI, NT-proBNP and H-FABP are not suitable as an early biomarker for this kind of cardiac dysfunction. For septic rats, DOB treatment in the presence of intrinsic myocardial dysfunction neither improves myocardial function nor attenuates myocardial inflammation and cardiomyocyte apoptosis at the later stage of sepsis.

Background

Sepsis,the life-threatening organ dysfunction induced by a dysregulated body response to infection, is a leading cause of critical illness and hospital mortality worldwide [1]. Myocardial dysfunction is a common complication in septic patients, about 50% of patients with severe sepsis and septic shock may exhibit pronounced myocardial dysfunction [2], which is associated with a higher mortality [3]. According to the current evidence, the pathogenesis of septic myocardial dysfunction involves a complex interaction of many factors, including inflammatory cytokines, apoptosis and neuroimmunomodulation [4–7]. For example, myocardial inhibitory factors, such as tumor necrosis factor (TNF) - α and interleukin (IL) -1β, could mediate myocardial dysfunction in experimental sepsis models [4]; Lipopolysaccharide (LPS) and cecal ligation and puncture (CLP) were found to induce myocardial vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 expression, blockade of VCAM-1 or ICAM-1 ameliorated myocardial dysfunction induced by sepsis [8, 9]; LPS also activated myocardial caspase-3 and apoptosis, broad-spectrum caspase inhibitors or caspase-3 inhibitors not only reduced LPS-induced myocardial caspase activation and nuclear apoptosis, but also improved LPS-evoked myocardial dysfunction[10, 11]. Although a large number of experimental studies have focused on myocardial dysfunction and associated mechanisms during sepsis over the last 50 years, pathophysiology of septic myocardial dysfunction is not completely understood and it remains a clinical enigma. The majority of clinical studies determined ventricular function in a global manner using indices, such as cardiac index and ejection fraction (EF) [4, 5, 12]. Indeed, left ventricular EF is a load-dependent index, which reflects the coupling between left ventricular contractility and afterload, rather than the left ventricular intrinsic myocardial contractile function. In sepsis, when the afterload is severely decreased due to reduced systemic resistance, left ventricular EF may be normal, despite seriously depressed left ventricular intrinsic contractility [5, 12, 13]. As a result of these limitations, the time pattern of intrinsic myocardial dysfunction progression during sepsis still needs clarification. Although recent great advancements have been made in echocardiography, by which the myocardial dysfunction is unmasked even in the presence of preserved left ventricular EF [14], the clinical relevance of intrinsic myocardial dysfunction is also still underestimated and no specific effective therapies for septic myocardial dysfunction exist.

In 2016, Surviving Sepsis Campaign Guidelines recommended that dobutamine, a β_1-adrenoceptor (AR) agonist, was the first-line inotrope for septic patients with low cardiac output despite adequate fluid resuscitation and the use of vasopressors [15]. However, recent studies have shown that dobutamine may increase myocardial oxygen consumption and the incidence of arrhythmias events in sepsis [16]. Furthermore, our recent studies found that exhaustion of cardiac norepinephrine or blockade of β_1-AR almost completely inhibited LPS-induced myocardial apoptosis [17], whereas activation of β_1-AR promoted LPS-induced cardiomyocyte apoptosis [18]. Evidently, there is no direct evidence to demonstrate if dobutamine treatment in the presence of intrinsic myocardial dysfunction improves intrinsic myocardial function at the later stage of sepsis.

Therefore, the purpose of the present study was to establish a rat polymicrobial sepsis model by the CLP approach and to determine: 1) when intrinsic myocardial systolic and diastolic dysfunction occurred

during sepsis, 2) and whether treatment with dobutamine in the presence of intrinsic myocardial dysfunction affected intrinsic myocardial function, cardiac inflammation and apoptosis at the later stage of sepsis.

Methods

Experimental animals

Male Sprague-Dawley (SD) rats, 8–10 weeks old (weight 250-300 g), were obtained from the medical laboratory animal center of Guangzhou University of Chinese Medicine and fed in the specific-pathogen-free laboratory environment for at least 10 days, in the conditions of temperature (24˚C), humidity (48%), and 12 h light/dark circadian cycle. All studies were conducted in compliance with the guide for the Care and Use of Laboratory animals published by US national institutes of health, and approved by the Animal Care and Use Committee at Jinan University. All efforts were made to minimize the number of rats used and their suffering.

Sepsis model

The model of sepsis was induced by CLP, as described previously [19]. Briefly, rats were anesthetized with isoflurane inhalation (3% isoflurane in 97% O_2) and then fixed on the operating table. A small midline abdominal surgical incision was performed under sterile conditions. The cecum was exposed and tightly ligated at 1.5 cm length of the distal cecum using a 4.0-silk suture, and then punctured once with an 18-gauge needle following with carefully squeezing to extrude a small amount of bowel content through the puncture site. Then, the cecum was repositioned into the abdominal cavity and the surgical incision was closed layer by layer. Sham-operated rats were performed using the same surgical procedure but without ligation or puncture of the cecum. Meanwhile, the catheters full of heparinized saline (50 IU heparin/ml) was inserted the right jugular vein for drug administration [20]. The normal saline (3 ml/100 g) were administered subcutaneously for post-surgery fluid resuscitation immediately after surgery, and buprenorphine (0.05 mg/kg body weight) was injected subcutaneously immediately and 12 h separately after CLP. All rats had free access to food and water when they were returned to their cages after recovery from anesthesia.

Experimental design

Firstly, male SD rats were randomly divided into sham and CLP groups. The survival rates were recorded for 10 days after CLP or sham operation. In a separate experiment, at 6 h, 9 h, 12 h after CLP or sham surgery, the rat hearts were harvested,the intrinsic myocardial functions were determined by the Langendorff perfusion system and left ventricle tissue was collected after heart perfusion. Meanwhile, the lungs were removed and the lung wet-dry weight (W/D) ratios were calculated, the serum inflammatory cytokine, cardiac troponin I (cTnI), N-terminal pro-brain natriuretic peptide (NT-proBNP) and heart-type fatty acid-binding protein (H-FABP) concentrations, alanine aminotransferase (ALT) and

aspartate aminotransferase (AST) activities as well as blood urea nitrogen (BUN) and creatinine (Cr) contents were analyzed.

Secondly, male SD rats were randomly divided into six groups, sham, CLP, CLP + 5 μg/kg DOB, CLP + 10 μg/kg DOB, sham + 5 μg/kg DOB and sham + 10 μg/kg DOB groups. At 6 h after CLP or sham operation, DOB (5 or 10 μg/kg/min, dobutamine hydrochloride, Sigma, USA) or normal saline (NS) was administered by the jugular vein for 2 h. At 20 h after sham operation or CLP surgery, the rat hearts were harvested and the intrinsic myocardial functions were determined by the Langendorff apparatus, meanwhile the serum samples were collected for enzyme-linked immunosorbent assay (ELISA) analysis. After heart perfusion, the left ventricular tissues were fixed in 4% paraformaldehyde for terminal deoxynucleotidy1 transferase dUTP nick-end labeling (TUNEL) assay, the total protein from the left ventricular tissues were extracted for Western blotting. Furthermore, serum and cardiac TNF-α, IL-6 and IL-1β were detected at 20 h after CLP or sham surgery by ELISA in a separate experiment.

Lastly, rats were randomly divided into five groups, sham, CLP group, CLP + 2.5 μg/kg DOB, CLP + 5 μg/kg DOB and CLP + 10 μg/kg DOB groups. At 6 h after sham operation or CLP, DOB (2.5, 5 or 10 μg/kg/min, dobutamine hydrochloride, Sigma, USA) or normal saline was administered by the jugular vein for 2 h. The survival rates were recorded for 10 d after CLP or sham operation. At 20 h after CLP exposure, serum IL-10 concentration was determined by ELISA in a separate experiment.

Langendorff perfusion

Male SD rats were anaesthetized with 3% isoflurane, and the myocardial functions were measured by modified method as described previously [21]. The hearts were rapidly harvested and immediately transferred to an ice-cold Krebs solution followed by cannulating the aorta. Then, the hearts were mounted in the Langendorff system (AD Instruments, USA) and subjugated to retrograde perfusion at 10 mL/min constant flow with modified warm Krebs-Henseleit solution (37°C), which consisted of NaCl 6.896 g / L, KCl 0.35 g / L, $MgSO_4$ 0.296 g / L, KH2PO_2 0.16 g / L, $NaHCO_3$ 2.1 g / L, glucose 2.18 g / L, $CaCl_2$ 0.277 g / L and equilibrated with 95% O_2 and 5% CO_2 at a pH of 7.35. A fluid-filled latex balloon was inserted into the left ventricle, and the initial end-diastolic pressure was adjusted at 4–10 mmHg quickly. After a 10 min stabilization period, myocardial functions, including left ventricular developed pressure, as well as the maximal rate of left ventricular pressure rise and fall (± dP/dt), were continuously recorded for up to 40 min by BL-420 F bio-transduction system.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay

The total RNA of left ventricular tissues was extracted using trizol reagent following the manufacturer's instructions. Prime Script RT Reagent Kit (RR047A, Takara) was used to make cDNA according to the manufacturer's instructions. The qPCR on Lightcycler®480 system (LC480, Roche, USA) was performed using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (RR820A, Takara) with the amplification program offered in accordance with the manufacturer's instruction. The sequences of primers, including

glyceraldehyde – 3-phosphate dehydrogenase (GAPDH) (forward 5'-AGGACCAGGTTGTCTCCTGT-3', reverse 5-'CCATGTAGGCCATGAGGTCC-3'), ICAM-1 (forward 5'-GCAGGTGAACTGCTCTTCCT-3', reverse 5'-GTCTTCCCCAATGTCGCTCA-3'), VCAM-1 (forward 5'-CGGAGCCTCAACGGTACTTT-3', reverse 5'-GCAAGTCAGGAGCATGGAGT-3') and TNF-α (forward 5'-CGTCAGCCGATTTGCCATTT-3', reverse 5'-TCCCTCAGGGGTGTCCTTAG-3') were synthesized by the Thermofisher scientific company. The relative quantification of the target genes were calculated and normalized to the expression of GAPDH.

Western blotting

The protein specimen of left ventricular tissues were extracted on ice using RIPA lysis buffer containing protease inhibitors according to the manufacturer's instructions. Then, the samples were centrifugation at 12000 rpm for 10 min at 4 ℃. Protein concentration was measured using Pierce™ BCA Protein Assay Kit (23225, Thermofisher). Equal volume of protein (10 μg) were loaded and separated by running 10% SDS-PAGE gels, and then transferred to PVDF membranes. The membranes were blocked with 5% non-fat dry milk at room temperature for 1 h, and then incubated with primary antibodies against GAPDH (2118S, Cell Signaling Technology), VCAM-1 (ab115135, Abcom), cleaved caspase-3 (9664S, Cell Signaling Technology), caspase-3 (9665S, Cell Signaling Technology) for overnight at 4 ℃. The membranes were washed three times for 10 min in 1x TBST and then incubated with the respective peroxidase labeled secondary antibodies at room temperature for 1 h. The membranes were then washed with 1X TBST for 3 times and developed using the Clarity Western ECL substrate. Finally, the density of the bands was quantified.

Enzyme-linked immunosorbent assay (ELISA).

The levels of TNF-α, IL-1β and IL-6 in serum and myocardial as well as serum IL-10 were detected using ELISA kits from R&D systems (USA). The levels of serum cTnI, heart-type fatty acid-binding protein (H-FABP) were measured using ELISA kits from Life Diagnostics (USA). The serum N-terminal pro-brain natriuretic peptide (NT-proBNP) levels were determined using the specific rat quantikine ELISA kits from Cloud-Clone Corp (China), according to the manufacture's protocol.

TUNEL assay

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL) assay was performed on cardiac tissue sections (4 μm) to detected cardiomyocyte apoptosis according to the *in situ* cell death detection kit 's instructions (11684817910, Roche). Briefly, the cardiac tissues were fixed with 4% paraformaldehyde at 4℃ for overnight, and then placed in 20%, 30%, 40% sucrose in PBS for gradient dehydration. After washing in 1X TBST, the sections were incubated with primary antibodies against cardiac troponin I (1:200, abcam, USA) at 4℃ for overnight, and then with prepared TUNEL reaction mixture in the dark at 37℃ for 1 h. The sections were rinsed with 1X TBST and incubated with fluorescent secondary antibody (1: 200) at room temperature for 1 h in the dark. Finally, the tissue sections were incubated with DAPI (1:150) for 15 min and mounted with coverslips. Then, the sections were visualized with laser-scanning confocal microscopy. The TUNEL-positive cells were determined by green staining in the nucleus of apoptotic cells.

Statistical analysis

Quantitative data were expressed as the mean ± standard error of the mean (SEM) and analyzed using the statistical software SPSS 20.0. The statistically significant difference was used by a two-tailed independent Student's *t*-test for two groups, the One-Way ANOVA test followed by Bonferroni post hoc test was performed to analyse the normally distributed data for the comparisons among the groups and non-parametric Mann-Whitney U tests were used for the data that was not normally distributed. The survival rate of rats was analyzed by Kaplan-Meier survival analysis with log-rank test. Statistical significance was accepted at $p < 0.05$.

Results

The intrinsic myocardial dysfunction occurred at 6 h after CLP surgery in septic rats.

We initially ascertained when intrinsic myocardial dysfunction was present in rats with CLP-induced sepsis. As shown in Fig. 1A, the mortality rate of septic rats was 70% on day 10 after CLP surgery. In order to determine left ventricular intrinsic systolic and diastolic function, the Langendorff technique of isolated heart perfusion was performed. The results revealed that left ventricular ± dP/dt markedly decreased in septic rats at 6 h, 9 h and 12 h after CLP exposure compared to sham-operated rats ($P < 0.05$) (Fig. 1B and 1C). The activities of serum ALT and AST were increased at 9 h and 12 h after CLP surgery in CLP rats compared with sham group ($P < 0.05$) (Fig. 1D and 1E). However, there was no significant difference in serum ALT and AST activities at 6 h after surgery between CLP rats and sham-operated rats, sham-operated and CLP rats did not substantially differ in serum BUN and Cr concentrations as well as lung W-D ratios at 6 h, 9 h and 12 h after surgery ($P > 0.05$) (Fig. 1D-1H). These results indicate that left ventricular intrinsic systolic and diastolic dysfunction are present at 6 h after CLP surgery in CLP rats, which is earlier than hepatic and renal dysfunction as well as lung edema in this condition.

Cardiac inflammation and injury in CLP rats

As mentioned above, myocardial TNF-α and adhesion molecules, such as ICAM-1and VCAM-1, contribute to the sepsis-induced myocardial dysfunction [4–8]. Real-time PCR and Western blot analyses showed that CLP rats had marked elevations in cardiac levels of TNF-α, ICAM-1 and VCAM-1 mRNAs as well as VCAM-1 protein at 6–12 h after CLP-induced sepsis compared with sham-operated controls, respectively ($P < 0.05$, Fig. 2A-2D). Additionally, greatly increased TNF-α concentration in plasma was observed at 6–12 h after CLP surgery in septic rats in comparison with sham-operated control animals (Fig. 2E). However, there was no significant difference in the levels of cTnI, NT-proBNP and H-FABP in plasma, clinical markers of cardiac injury, between CLP and sham- operated control rats at 6 h, 9 h and 12 h after surgery (Fig. 2F-2H).

DOB did not affect intrinsic myocardial dysfunction at the late stage of sepsis in CLP rats

Surviving sepsis campaign guidelines suggested administering DOB in the presence of myocardial dysfunction. Sakai M, et al. demonstrated that intravenous administration of 10 μg/kg dobutamine had a marked rapid positive inotropic response on the sham-operated mouse heart, which lasted for 5 min, but not on CLP-treated mouse heart at 18 h after CLP exposure [22]. However, the long-term effect of dobutamine on cardiac dysfunction in sepsis remains unclear. We found CLP induced a significant intrinsic myocardial dysfunction at 6 h after CLP surgery. Therefore, we further observed the effect of DOB administered at 6 h after CLP induction on intrinsic myocardial function at the late stage of sepsis in septic rats with myocardial dysfunction. The CLP significantly reduced ± dP/dt of the left ventricle at 20 h after CLP compared to sham group, but intravenous administration of DOB (5, 10 μg/kg) at 6 h after CLP induction did not increase left ventricular ± dP/dt at 20 h after CLP exposure in septic rats. In addition, intravenous administration of DOB (5, 10 μg/kg) at 6 h after sham surgery did not affect left ventricular ± dP/dt at 20 h after surgery in sham- operated control rats (Fig. 3A, 3B).

DOB did not affect cardiac inflammation and myocardial apoptosis in CLP rats with myocardial dysfunction.

Inflammatory cytokines, such as TNF-α, IL-1β and IL-6, as well as myocardial apoptosis contribute to the sepsis-induced myocardial dysfunction. We further investigated the long-term effect of DOB administered at 6 h after CLP induction on cardiac cytokines and myocardial apoptosis at 20 h after CLP surgery in septic rats. Compared with sham group, CLP markedly increased the concentrations of serum and cardiac TNF-α, IL-1β and IL-6 as well as myocardial caspase-3 activation and apoptosis at 20 h after CLP, all of which were not affected by treatment with DOB (5, 10 μg/kg) at 6 h after CLP (Fig. 4, 5).

DOB (10 μg/kg) increased serum IL-10 concentration and improved survival in CLP rats.

Intriguingly, serum IL-10 concentration was significantly increased at 20 h after CLP and further elevated by DOB (10 μg/kg) administered at 6 h after CLP in septic rats ($P < 0.05$), which was not the case in septic rats treated with 5 μg/kg DOB (Fig. 6B). Importantly, DOB (10 μg/kg) administered at 6 h after CLP improved survival in CLP rats compared with CLP group ($P < 0.05$) (Fig. 6A).

Discussion

Dobutamine has been used in septic patients with low cardiac output for many years as a first-line therapy recommended by the Surviving Sepsis Campaign guidelines [15]. However, clinical outcome estimation is limited for advisability of the usefulness of dobutamine in the treatment of septic shock patients in the presence of intrinsic myocardial dysfunction. Recently, Sakai M, et al. found that positive inotropic action of dobutamine was markedly impaired in sepsis due to increased cAMP breakdown caused by myocardial phosphodiesterase 4 upregulation, intravenous injection of dobutamine (0.01 mg/kg) had a significant fast positive inotropic effect on the heart in sham-operated mice, but no fast positive inotropic response in CLP mice [22]. On the other hand, it has been demonstrated that

activation of β_1- AR promoted LPS-induced cardiomyocyte apoptosis [18]. Therefore, it is necessary to further observe the effect of dobutamine, a β_1- AR agonist, on intrinsic myocardial function during sepsis.

In order to investigate the long-term effect of dobutamine on intrinsic myocardial dysfunction in sepsis, we first determined when intrinsic myocardial systolic and diastolic dysfunction occurred during severe sepsis in the present study. It is well known that left ventricular ejection fraction is a load-dependent indicator, which did not accurately reflect the intrinsic contraction and diastolic function during sepsis [13]. We utilized the Langendorff perfusion system to measure the intrinsic myocardial function that less affected by vascular loading conditions in sepsis. In this study, we found that the mortality rate was 70% in CLP rats on day 10 after CLP induction. In this condition, the CLP rats had significant intrinsic myocardial dysfunction at 6 h after CLP exposure. However, the liver dysfunction occurred at 9 h after CLP induction, but the serum Cr, BUN and lung W-D ratios did not change at 6 h, 9 h and 12 h after CLP surgery. These data indicated that severe CLP rats had intrinsic myocardial dysfunction in the early stage of sepsis, which was earlier than the dysfunction of liver and kidney and lung edema in the severe CLP rats.

It is well known that the production of cytokines (e.g. TNF-α) and adhesion molecules (e.g. ICAM-1 and VCAM-1) are significantly increased in sepsis-induced myocardial dysfunction, inhibition of TNF-α, ICAM-1 or VCAM-1 improves left ventricular function in sepsis [7, 8, 23, 24]. In the present study, we observed that serum TNF-α level, the mRNA expression of cardiac TNF-α, ICAM-1 and VCAM-1, and VCAM-1 protein levels were significantly increased in CLP rats at 6 h, 9 h, and 12 h after CLP. These results further demonstrated these inflammatory molecules contributed to pathogenesis of intrinsic myocardial dysfunction at 6–12 h after CLP. It was reported that some serum myocardial injury markers, such as NT-proBNP, cTnI and H-FABP, might reflect sepsis-induced myocardial dysfunction [25–28]. Sakai M, et al. demonstrated that serum cardiac troponin-I (cTnI) levels were significantly increased at 18 h after CLP induction in septic mice with cardiac dysfunction [22]. In this study, we found that the levels of serum cTnI, NT-proBNP and H-FABP did not increase at 6–12 h after CLP in CLP rats compared with sham-operated rats. These results indicate serum cTnI, NT-proBNP and H-FABP are not suitable as early biomarkers for this kind of intrinsic myocardial dysfunction in sepsis.

As mentioned above, DOB was suggested to be administered in the presence of myocardial dysfunction in septic patients [15]. According to our study that showed intrinsic myocardial dysfunction occurred at 6 h after CLP exposure, DOB was injected at 6 h after CLP induction in CLP rats. We found that DOB treatment (5 or 10 μg/kg/min for 2 h) had no significant impact on the intrinsic contraction and diastolic dysfunction at 20 h after CLP exposure in CLP rats. We further found that DOB treatment (5 or 10 μg/kg/min for 2 h) did not change the levels of serum and myocardial TNF-α,IL-1β and IL-6 in CLP rats. In addition, our previous study confirmed that DOB promoted LPS-induced cardiomyocyte apoptosis *in vitro* through activating cAMP-dependent protein kinase and enhancing calmodulin-dependent protein kinase II and IκBα phosphorylation [18]. However, in the present study, we found that intravenous administration of DOB have no significant impact on cardiac caspase-3 activation and cardiomyocyte apoptosis 20 h after CLP surgery.

It was reported that pretreatment with reserpine that exhausts cardiac norepinephrine without affecting the circulating norepinephrine concentration significantly inhibited cardiomyocyte apoptosis in septic rats [29] and β_1-$_{AR}$ antagonist attenuated LPS-caused cardiomyocyte apoptosis [17]. These results indicate that activation of myocardial β_1-$_{AR}$ by norepinephrine derived from cardiac sympathetic nerve, rather than circulating norepinephrine, promoted cardiomyocyte apoptosis in sepsis. This may explain why intravenous administration of DOB did not affect cardiomyocyte apoptosis in sepsis in the present study. In addition, the previous studies demonstrated that the downregulation /desensitization of β_1-AR signal and decreased response of myofilament to Ca^{2+} occurred in sepsis [30–33]. These findings may also explain why DOB treatment after CLP surgery did not affect cardiomyocyte apoptosis and intrinsic myocardial dysfunction in sepsis.

Furthermore, we found that intravenous dobutamine at a dose up to 10 μg/kg significantly improved the survival in septic rats with myocardial dysfunction. This finding dovetails well with other studies which demonstrated that DOB had improved the survival in patients with septic shock and animal models of sepsis [34, 35]. We also found intravenous dobutamine at a dose of 10 μg/kg markedly increased serum IL-10 level in CLP rats. It has been demonstrated that activation of β receptors amplifies the release of IL-10 by LPS-induced macrophages, IL-10-deficient mice increases mortality in Escherichia coli-treated mice [36, 37], and interleukin-10 administered after the onset of CLP protected against the lethality of septic rats [38]. Thus, the reason that treatment with DOB a dose of 10 μg/kg improved the survival may be related with the increased levels of IL-10 in CLP rats.

Conclusions

In the present study, we demonstrated that intrinsic myocardial dysfunction occurred earlier than hepatic and renal dysfunction in severe sepsis and serum cTnI, NT-proBNP and H-FABP were not suitable as early biomarkers for the intrinsic cardiac depression in sepsis. Treatment with DOB in the presence of intrinsic myocardial dysfunction (at 6 h after CLP) did not attenuate the intrinsic myocardial dysfunction, inflammation and cardiomyocyte apoptosis at the later stage (at 20 h after CLP) of septic rats. These results strongly suggest that it is necessary to reevaluate advisability of the usefulness of dobutamine and to seek a new inotrope for the treatment of septic patients with low cardiac output.

Abbreviations

ALT
alanine aminotransferase; AR:adrenoceptor; AST:aspartate aminotransferase; BUN:blood urea nitrogen; CLP:cecal ligation and puncture; Cr:creatinine; cTnI:cardiac troponin I; DOB:Dobutamine; EF:ejection fraction; ELISA:enzyme-linked immunosorbent assay; GAPDH:glyceraldehyde-3-phosphate dehydrogenase; H-FABP:heart-type fatty acid-binding protein; ICAM:intercellular adhesion molecule; IL:interleukin; LPS:Lipopolysaccharide; NT-proBNP:N-terminal pro-brain natriuretic peptide; RT-qPCR:reverse transcription-quantitative polymerase chain reaction; SD:Sprague-Dawley; TNF:tumor

necrosis factor; TUNEL:terminal deoxynucleotidy1 transferase dUTP nick-end labeling; VCAM:vascular cell adhesion molecule

Declarations

Availability of data and materials

All materials are commercially available, and data in this study are available per the open access policy.

Ethics approval

All animal studies were conducted in compliance with the guide for the Care and Use of Laboratory animals published by US national institutes of health, and approved by the Animal Care and Use Committee at Jinan University.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Funding

This study was supported by grants from the National Natural Science Foundation of China (81372028,81670359).

Authors' contributions

XXT conducted the study, analyzed the data, and drafted the manuscript. YQX made ELISA analysis and prepared the manuscript. DXL analyzed the data. HDW designed the study, analyzed the data and made revisions throughout the manuscript. The remaining authors contributed for the experiments and acquisition of data. All authors read and approved the final manuscript to be published.

Acknowledgments

We thank the National Natural Science Foundation of China for supporting this study.

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Jama. 2016;315(8):801-10.
2. Vallabhajosyula S, Kumar M, Pandompatam G, Sakhuja A, Kashyap R, Kashani K, et al. Prognostic impact of isolated right ventricular dysfunction in sepsis and septic shock: an 8-year historical cohort study. Ann Intensive Care. 2017;7(1):94.
3. Sanfilippo F, Corredor C, Arcadipane A, Landesberg G, Vieillard-Baron A, Cecconi M, et al. Tissue Doppler assessment of diastolic function and relationship with mortality in critically ill septic patients: a systematic review and meta-analysis. Br J Anaesth. 2017;119(4):583-94.
4. Antonucci E, Fiaccadori E, Donadello K, Taccone FS, Franchi F, Scolletta S. Myocardial depression in sepsis: from pathogenesis to clinical manifestations and treatment. J Crit Care. 2014;29(4):500-11.
5. Lv X, Wang H. Pathophysiology of sepsis-induced myocardial dysfunction. Mil Med Res, 2016;3:30.
6. Liu YC, Yu MM, Shou ST, Chai YF. Sepsis-Induced Cardiomyopathy: Mechanisms and Treatments. Front Immunol. 2017;8:1021.
7. Walley KR. Sepsis-induced myocardial dysfunction. Curr Opin Crit Care. 2018;24(4):292-9.
8. Raeburn CD, Calkins CM, Zimmerman MA, Song Y, Ao L, Banerjee A, et al. ICAM-1 and VCAM-1 mediate endotoxemic myocardial dysfunction independent of neutrophil accumulation. Am J Physiol Regul Integr Comp Physiol. 2002;283(2):R477-86.
9. Ma H, Wang X, Ha T, Gao M, Liu L, Wang R, et al. MicroRNA-125b prevents cardiac dysfunction in polymicrobial sepsis by targeting TRAF6-mediated nuclear factor κB activation and p53-mediated apoptotic signaling. J Infect Dis. 2016;214(11):1773-83.
10. Fauvel H, Marchetti P, Chopin C, Formstecher P, Nevière R. Differential effects of caspase inhibitors on endotoxin-induced myocardial dysfunction and heart apoptosis. Am J Physiol Heart Circ Physiol. 2001;280(4):H1608-14.
11. Neviere R, Fauvel H, Chopin C, Formstecher P, Marchetti P. Caspase inhibition prevents cardiac dysfunction and heart apoptosis in a rat model of sepsis. Am J Respir Crit Care Med. 2001;163(1):218-25.
12. Zaky A, Gill EA, Lin CP, Paul CP, Bendjelid K, et al. Characteristics of sepsis-induced cardiac dysfunction using speckle-tracking echocardiography: a feasibility study. Anaesth Intensive Care. 2016;44(1):65-76.
13. Zaky A, Deem S, Bendjelid K, Treggiari MM. Characterization of cardiac dysfunction in sepsis: an ongoing challenge. Shock.2014;41(1):12-24.
14. Basu S, Frank LH, Fenton KE, Sable CA, Levy RJ, Berger JT. Two-dimensional speckle tracking imaging detects impaired myocardial performance in children with septic shock, not recognized by conventional echocardiography. Pediatr Crit Care Med. 2012; 13(3):259-64.
15. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43(3):304-77.

16. Stratton L, Berlin DA, Arbo JE. Vasopressors and Inotropes in Sepsis. Emerg Med Clin North Am. 2017;35(1):75-91.
17. Wang Y, Yu X, Wang F, Wang Y, Wang YP, Li HM, et al. Yohimbine promotes cardiac NE release and prevents LPS-induced cardiac dysfunction via blockade of presynaptic alpha2A-adrenergic receptor .PloS one. 2013;8(5):e63622.
18. Wang Y, Wang Y, Yang D, Yu X, Li H, Lv X, et al. β-adrenoceptor stimulation promotes LPS-induced cardiomyocyte apoptosis through activating PKA and enhancing CaMKII and IκBα phosphorylation. Crit Care. 2015;19(1):76.
19. Chang R, Holcomb JB, Johansson PI, Pati S, Schreiber MA, Wade CE. Plasma resuscitation improved survival in a cecal ligation and puncture rat model of sepsis. Shock. 2018;49(1):53-61.
20. Feng J, Fitz Y, Li Y, Fernandez M, Cortes Puch I, Wang D, et al. Catheterization of the carotid artery and jugular vein to perform hemodynamic measures, infusions and blood sampling in a conscious rat model. J Vis Exp. 2015;(95):51881.
21. Watanabe M, Okada T. Langendorff perfusion method as an ex vivo model to evaluate heart function in rats. Methods Mol Biol. 2018;1816:107-16.
22. Sakai M, Suzuki T, Tomita K, Yamashita S, Palikhe S, Hattori K, et al. Diminished responsiveness to dobutamine as an inotrope in mice with cecal ligation and puncture-induced sepsis: attribution to phosphodiesterase 4 upregulation. Am J Physiol Heart Circ Physiol. 2017;312(6):H1224-37.
23. Peng T, Lu X, Lei M, Moe GW, Feng Q. Inhibition of p38 MAPK decreases myocardial TNF-alpha expression and improves myocardial function and survival in endotoxemia. Cardiovasc Res. 2003;59(4):893-900.
24. Vincent JL, Bakker J, Marécaux G, Schandene L, Kahn RJ, Dupont E. Administration of anti-TNF antibody improves left ventricular function in septic shock patients. Results of a pilot study. Chest. 1992;101(3):810-5.
25. Chen FC, Xu YC, Zhang ZC. Multi-biomarker strategy for prediction of myocardial dysfunction and mortality in sepsis. J Zhejiang Univ Sci B. 2020;21(7):537-48.
26. Zhen J, Li L, Yan J. Advances in biomarkers of myocardial injury in sepsis. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2018;30(7):699-702.
27. Fernandes CJ Jr, Akamine N, Knobel E. Cardiac troponin: a new serum marker of myocardial injury in sepsis. Intensive Care Med. 1999;25(10):1165-8.
28. Hoffmann U, Brueckmann M, Bertsch T, Wiessner M, Liebetrau C, Lang S, et al. Increased plasma levels of NT-proANP and NT-proBNP as markers of cardiac dysfunction in septic patients. Clin Lab. 2005;51(7-8):373-9.
29. Yu X, Wang Y, Yang D, Tang X, Li H, Lv X, et al. α2A-adrenergic blockade attenuates septic cardiomyopathy by increasing cardiac norepinephrine concentration and inhibiting cardiac endothelial activation. Sci Rep. 2018;8(1):5478.
30. Thangamalai R, Kandasamy K, Sukumarn SV, Reddy N, Singh V, Choudhury S, et al. Atorvastatin prevents sepsis-induced downregulation of myocardial β1-adrenoceptors and decreased cAMP

response in mice. Shock. 2014 ;41(5):406-12.

31. Silverman HJ, Penaranda R, Orens JB, Lee NH. Impaired beta-adrenergic receptor stimulation of cyclic adenosine monophosphate in human septic shock: association with myocardial hyporesponsiveness to catecholamines. Crit Care Med. 1993;21(1):31-9.
32. Tavernier B, Mebazaa A, Mateo P, Sys S, Ventura-Clapier R, Veksler V. Phosphorylation-dependent alteration in myofilament ca2+ sensitivity but normal mitochondrial function in septic heart. Am J Respir Crit Care Med. 2001 ;163(2):362-7.
33. avernier B, Li JM, El-Omar MM, Lanone S, Yang ZK, Trayer IP, et al. Cardiac contractile impairment associated with increased phosphorylation of troponin I in endotoxemic rats. FASEB J. 2001;15(2):294-6.
34. Cheng L, Yan J, Han S, Chen Q, Chen M, Jiang H, et al. Comparative efficacy of vasoactive medications in patients with septic shock: a network meta-analysis of randomized controlled trials. Crit Care. 2019;23(1):168.
35. Fink T, Heymann P, Taha-Melitz S, Taha A, Wolf B, Rensing H, et al. Dobutamine pretreatment improves survival, liver function, and hepatic microcirculation after polymicrobial sepsis in rat. Shock. 2013;40(2):129-35.
36. Sewnath ME, Olszyna DP, Birjmohun R, ten Kate FJ, Gouma DJ, van Der Poll T. IL-10-deficient mice demonstrate multiple organ failure and increased mortality during Escherichia coli peritonitis despite an accelerated bacterial clearance. J Immunol. 2001;166(10):6323-31.
37. Suberville S, Bellocq A, Fouqueray B, Philippe C, Lantz O, Perez J, et al. Regulation of interleukin-10 production by beta-adrenergic agonists. Eur J Immunol. 1996;26(11):2601-5.
38. Rongione AJ, Kusske AM, Kwan K, Ashley SW, Reber HA, McFadden DW. Interleukin-10 protects against lethality of intra-abdominal infection and sepsis. J Gastrointest Surg. 2000;4(1):70-6.

Figures

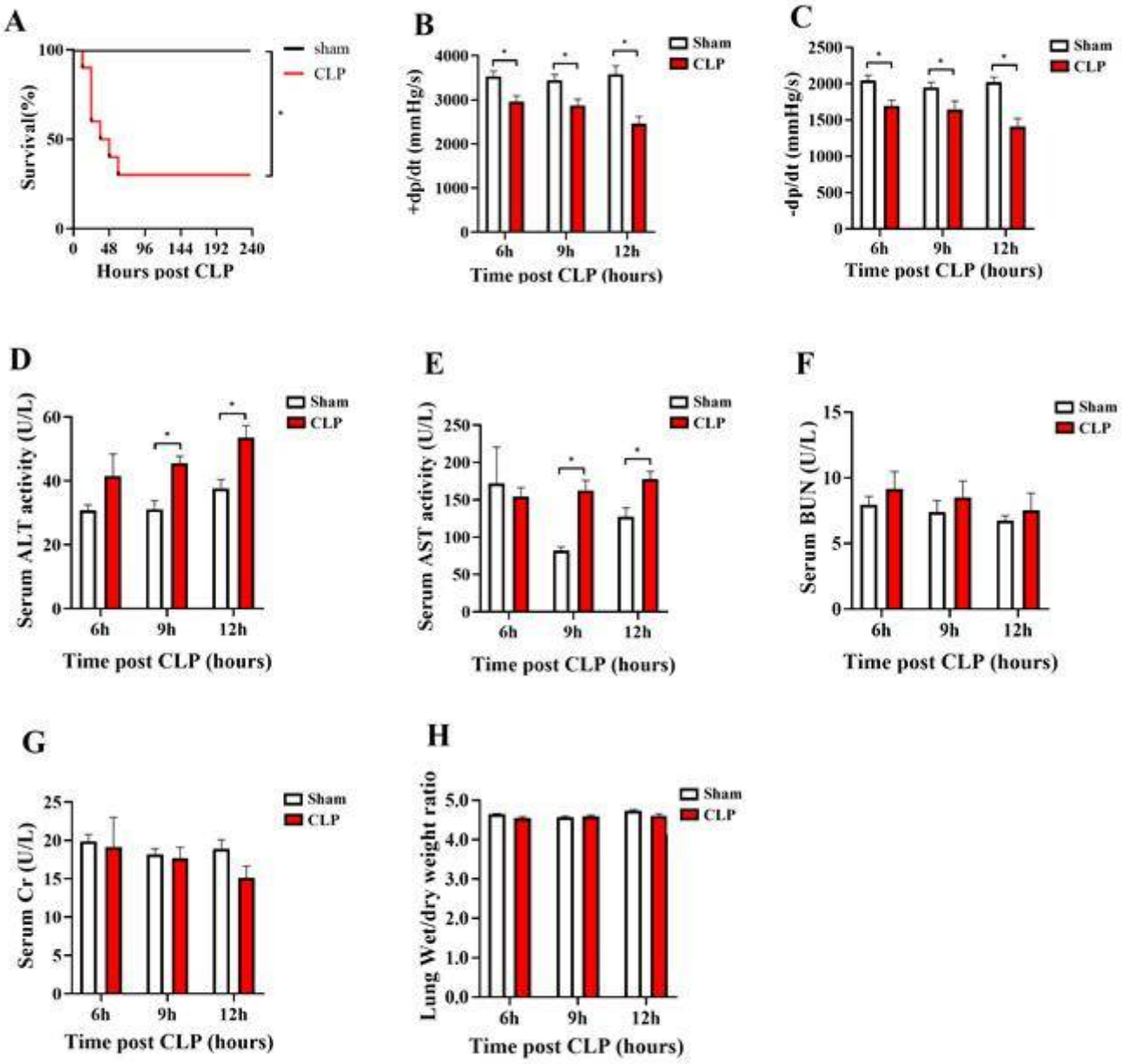

Figure 1

Survival curves of cecal ligation and puncture (CLP) rats as well as changes in cardiac, liver and renal function and lung wet-dry weight ratios at different time-point after CLP induction. A. Kaplan–Meier curves of CLP rats, male Sprague-Dawley rats were randomized to undergo CLP or sham surgery, the rats were monitored for lethality every 12 h for up to 240 h. n=10 in each group. *P<0.05. At 6 h, 9 h and 12 h after CLP exposure, left ventricular ±dP/dt were detected by Langendorff system (B, C), serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr), blood urea nitrogen (BUN) and lung wet-dry weight ratios were determined (D-H). n=5-10. *P<0.05.

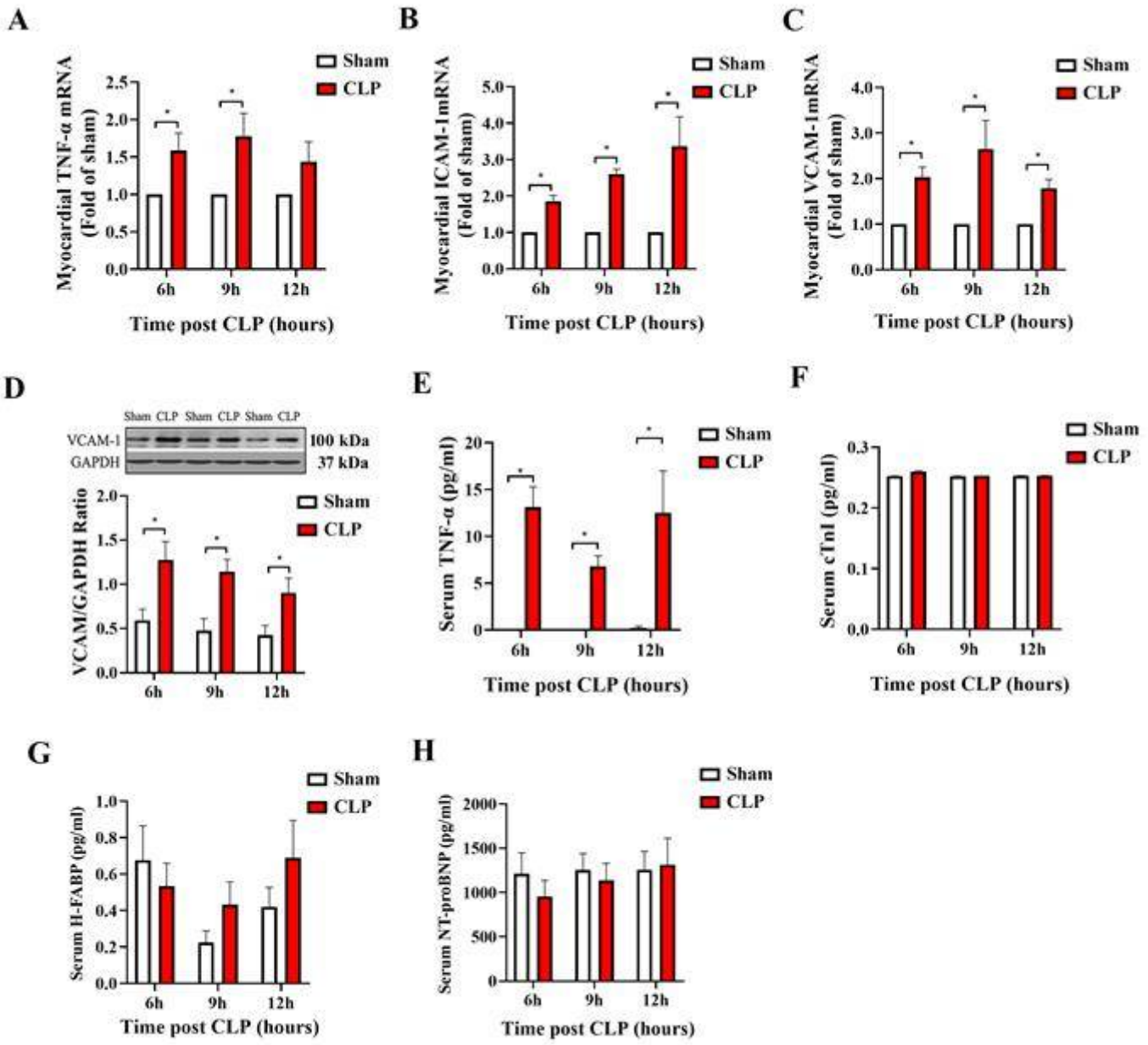

Figure 2

Cardiac inflammation as well as serum TNF-α and biomarker of cardiac injury in rats after CLP-induced sepsis. At 6 h, 9 h and 12 h after CLP or sham surgery, myocardial mRNA levels of TNF-α, ICAM-1 and VCAM-1 were quantified by RT-qPCR (A,B,C), myocardial VCAM-1 protein expression was detected by Western blot (D), serum TNF-α, cardiac troponin-I (cTnI), heart-type fatty acid-binding protein (H-FABP), N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations were also determined (E-H), respectively. n=5-10. *P<0.05.

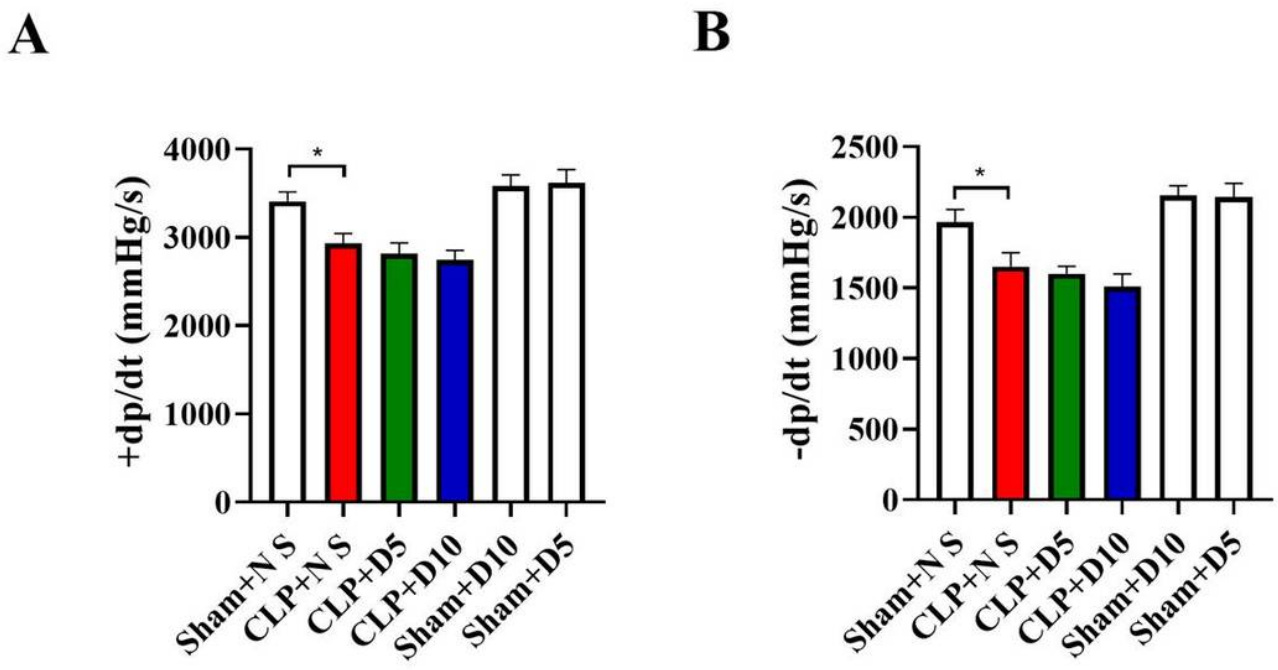

Figure 3

The long-term effect of dobutamine (DOB, 5, 10 μg/kg) administered at 6 h after CLP on cardiac dysfunction in septic rats. Sprague-Dawley rats were randomized to undergo CLP or sham surgery, DOB at a dose of 5 μg/kg (D5), 10 μg/kg (D10) or normal saline (NS) was administered intravenously at 6 h after CLP or sham surgery, left ventricular ±dP/dt were detected at 20 h after CLP or sham surgery by Langendorff system (A, B). n=8-10. *P<0.05.

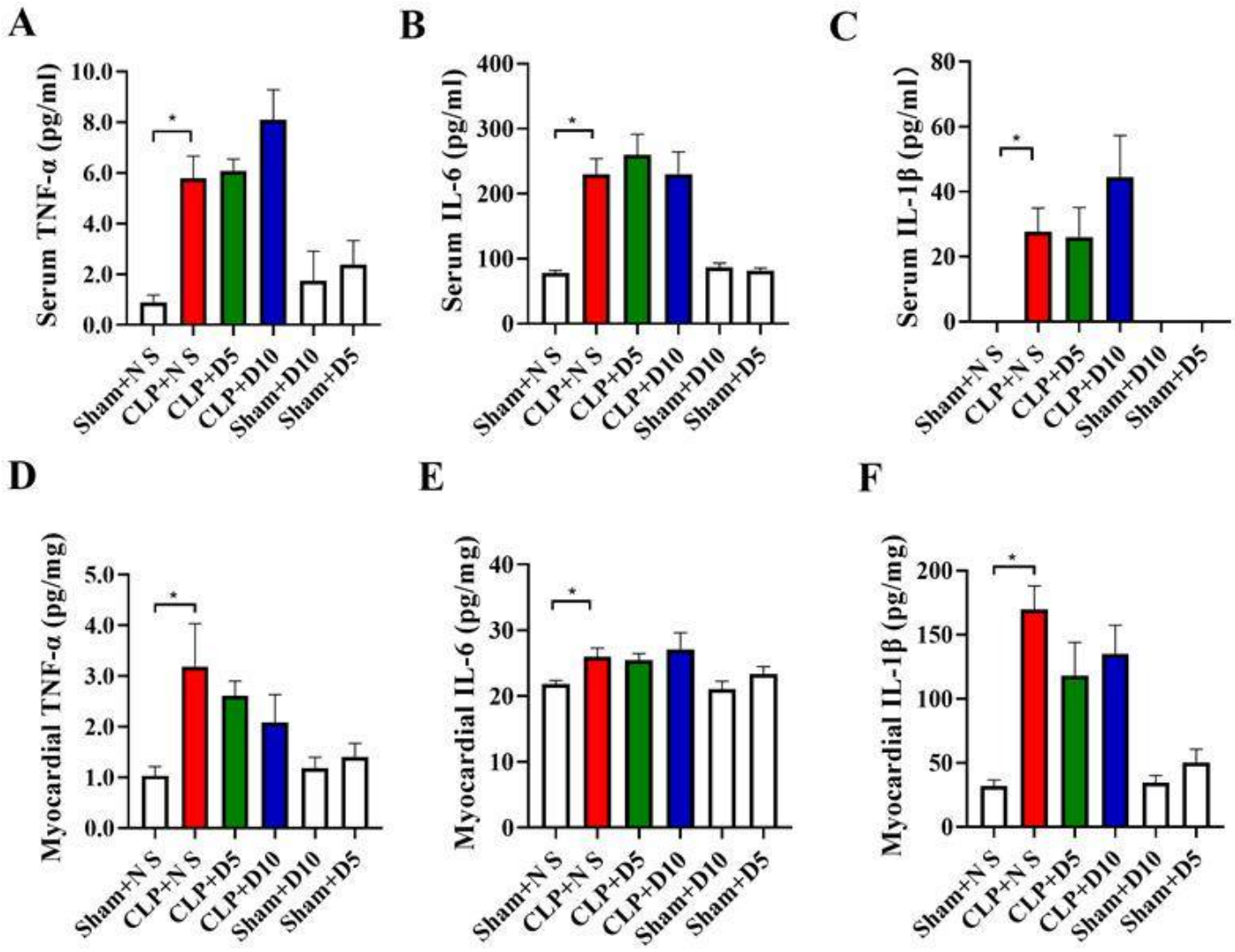

Figure 4

The long-term effect of dobutamine (DOB, 5, 10 □g/kg) administered at 6 h after CLP on serum and cardiac cytokines in septic rats. Sprague-Dawley rats were randomized to undergo CLP or sham surgery, DOB at a dose of 5 □g/kg (D5), 10 □g/kg (D10) or normal saline (NS) was administered intravenously at 6 h after CLP or sham surgery, serum (A-C) and cardiac (D-F) TNF-□, interleukin (IL)-6 and IL-1□ were detected at 20 h after CLP or sham surgery by ELISA. n=6-13. *P<0.05.

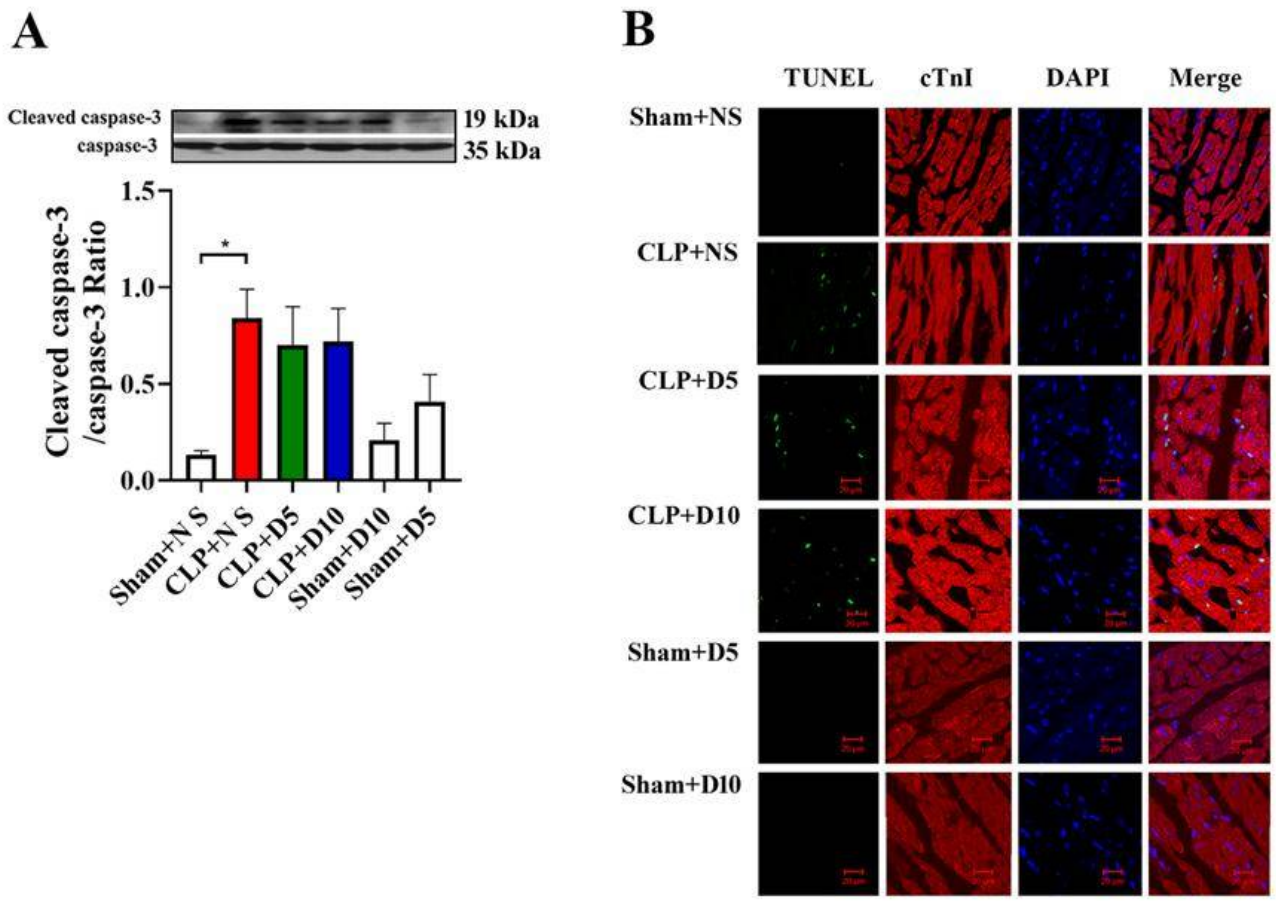

Figure 5

The effect of dobutamine (DOB, 5, 10 μg/kg) administered at 6 h after CLP on myocardial aopotosis in septic rats. Male Sprague-Dawley rats were randomized to undergo CLP or sham surgery, DOB at a dose of 5 μg/kg (D5), 10 μg/kg (D10) or normal saline (NS) was administered intravenously at 6 h after CLP or sham surgery. A. cardiac caspase-3 and cleaved caspase-3 were determined by Western blot, and myocardial apoptosis were detected by Terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL) assay at 20 h after CLP or sham surgery. n=5-10. *P<0.05. B. Photomicrographs illustrate representative TUNEL (green), cTnI (red) and DAPI (blue)-stained myocardial sections. Scale bar= 20 μm.

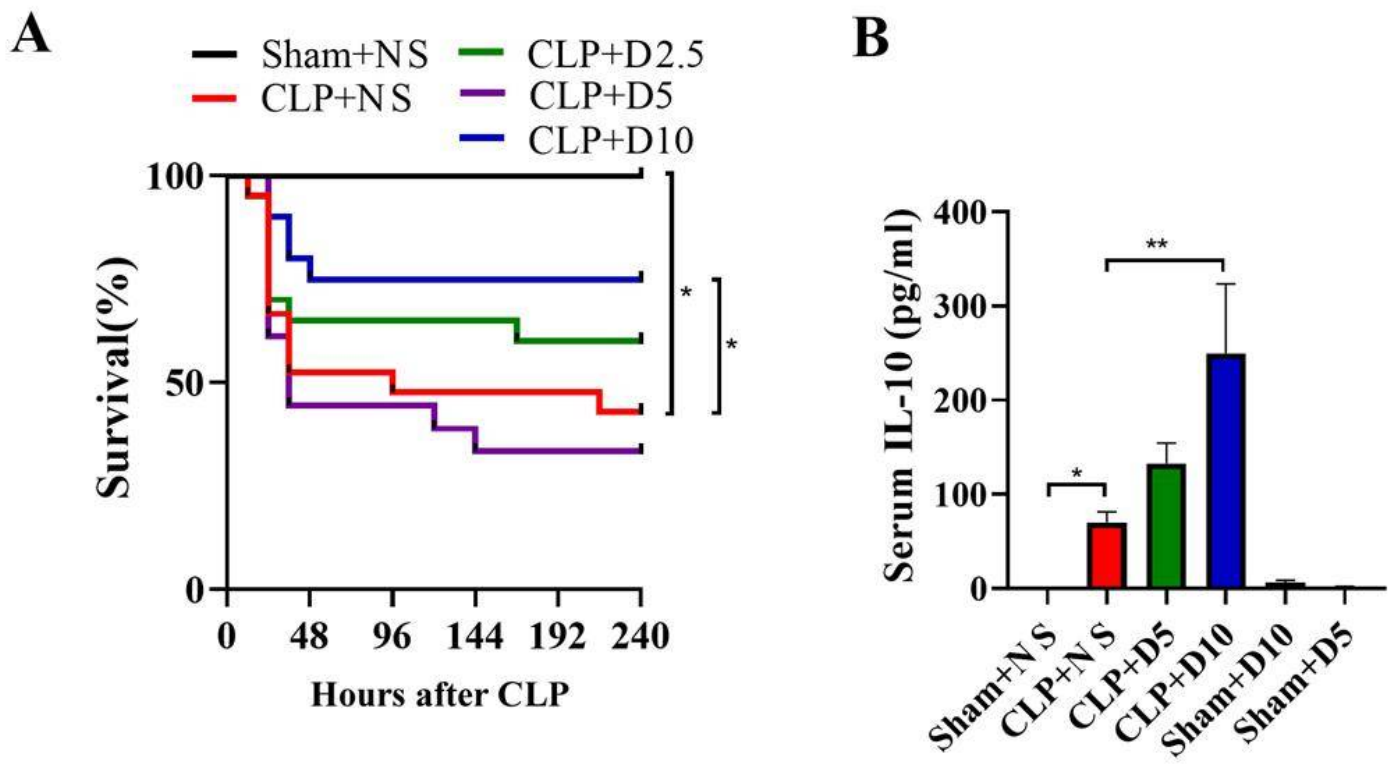

Figure 6

The effect of dobutamine (DOB, 2.5, 5, 10 μg/kg) administered at 6 h after CLP on survival and serum interleukin-10 (IL-10) level in septic rats. Male Sprague-Dawley rats were randomized to undergo CLP or sham surgery, DOB at a dose of 2.5 μg/kg (D2.5), 5 μg/kg (D5), 10 μg/kg (D10) or normal saline (NS) was administered intravenously at 6 h after CLP or sham surgery. A. The rats were monitored for lethality every 12 h for up to 240 h. n=19-22. *P<0.05. B. Serum IL-10 concentration was determined by ELISA at 20 h after CLP exposure. n=7-11. *P<0.05, **P<0.01.