Original Article

Cardioprotective effect of erythropoietin on sepsis-induced myocardial injury in rats

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BACKGROUND: Sepsis-induced myocardial injury is one of the major predictors of morbidity and mortality of sepsis. The cytoprotective function of erythropoietin (EPO) has been discovered and extensively studied. However, the cardioprotective effects of EPO on sepsis-induced myocardial injury in the rat sepsis model has not been reported.

METHODS: The rat models of sepsis were produced by cecal ligation and perforation (CLP) surgery. Rats were randomly (random number) assigned to one of three groups (n=8 for each group): sham group, CLP group and EPO group (1000 IU/kg erythropoietin). Arterial blood was withdrawn at 3, 6, 12, and 24 hours after CLP. cTnI, BNP, CK-MB, LDH, AST, TNF-α, IL-6, IL-10, and CRP were tested by the ELISA assay. Changes of hemodynamic parameters were recorded at 3, 6, 12, 24 hours after the surgery. Histological diagnosis was made by hematoxylin and eosin. Flow cytometry was performed to examine cell apoptosis, myocardium mitochondrial inner membrane potential, and NF-κB (p65). Survival rate at 7 days after CLP was recorded.

RESULTS: In the CLP group, myocardial enzyme index and inflammatory index increased at 3, 6, 12 and 24 hours after CLP compared with the sham group, and EPO significantly blocked the increase. Compared with the CLP group, EPO significantly improved LVSP, LV $+dp/dt_{max}$, LV $-dp/dt_{min}$, and decreased LVEDP at different time. EPO blocked the reduction of mitochondrial transmembrane potential, suppressed the cardiomyocyte apoptosis, inhibited the activation of NF-κB, and reduced the production of proinflmmatory cytokines. No difference in the survival rate at 7 days was observed between the CLP group and the EPO group.

CONCLUSION: Exogenous EPO has cardioprotective effects on sepsis-induced myocardial injury.

KEY WORDS: Sepsis; Sepsis-induced myocardial injury; Apoptosis; Chondriosome membrane potential; Nuclear factor κB p65; Erythropoietin; Inflammatory cytokines; Rat

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INTRODUCTION

Sepsis-induced myocardial injury is one of the major predictors of morbidity and mortality of sepsis. It is present in more than 40% of sepsis patients[1] and its appearance can increase the mortality rate up to 70%.[2] Norepinephrine and epinephrine improved global hemodynamics and myocardial function during experimental septic shock but epinephrine increased myocardial oxygen consumption, whereas phenylephrine decreased ventricular performance.[3] Moreover, numerous adverse effects of catecholamines on heart function have been reported, including tachycardia/tachyarrhythmia and myocardial stunning to necrosis and apoptosis.[4,5] Their net effects are still not clear if vasopressors are applied in sepsis or sepsis shock. Therefore, the development of new therapies for severe sepsis and septic shock is needed to relieve sepsis-induced myocardial dysfunction.

Erythropoietin (EPO) is a glycoprotein hormone whose primary role is to stimulate and maintain red cell production via inhibition of apoptosis in blast cells, thus regulating the balance between erythrocyte production and loss.[6] With the clinical potential role of EPO recognized, recombinant human EPO (rhEPO) is widely used in the treatment of anemia.[7] During the last decade, new properties of EPO other than erythropoietic, mainly cytoprotective, have been discovered and extensively studied.[8] A functional EPO receptor is found in the cardiovascular system, including endothelial cells and cardiomyocytes.[9] In animal studies, treatment with EPO during ischemia/reperfusion in the heart has been shown to limit the infarct size and the extent of apoptosis.[9] A recent study[10] have demonstrated therapeutic effects of EPO in murine models of endotoxin shock. However, the cardioprotective effects of EPO on sepsis-induced myocardial dysfunction or damage in the rat sepsis model have not been reported. The objectives of this study are to evaluate whether EPO exerts cardioprotective effects on the experimental rat sepsis model and the possible mechanisms.

METHODS

Sepsis models

Adult male Sprague Dawley rats (210–250 g) were provided by the Experimental Center of Hebei Medical University. The rats were allowed to acclimatize for at least 7 days after delivery and were maintained on 12 hours light-dark cycles with *ad libitum* food and water at all times. Cecal ligation was performed as previously described with some modifications.[11,12] In brief, the rats were anesthetized with chloral hydrate (Tianjin Kemiou Chemical Reagent Co. Ltd, China) (40 mg/kg i.p.). After shaving and cleaning the ventral abdominal wall with alcohol, a 15-mm midline abdominal incision was made to expose the cecum. The cecum was isolated carefully and then ligated at about 20% of the total length just below the ileocecal valve to avoid bowel obstruction. The cecum was punctured twice on the anti-mesenteric side with a sterile18-gauge needle and was gently squeezed to extrude the fecal material into the peritoneal cavity. The cecum was placed back in the abdomen, and the incision was closed in two layers with sutures. All rats were then resuscitated with subcutaneous normal saline 10 mL/kg immediately after surgery. The entire procedure was completed within 8 minutes. The arterial catheter was infused with normal saline at a rate of 0.5 mL/h. In the sham group, the cecum of each rat was exposed and the bowel was massaged as described above, but it was not ligated or punctured. All procedures involving the rats were conducted in accordance with the protocol approved by the committee on animal experimentation of Hebei Medical University.

Experimental protocols

Rats were randomly (random number) divided into three groups (8 rats for each group): 1) a sham group receiving an intraperitoneal injection of 1 mL of normal saline after sham operation; 2) a CLP group receiving an intraperitoneal injection of 1 mL of normal saline after CLP; 3) an EPO group receiving an intraperitoneal injection of 1000 IU/kg erythropoietin (Shanghai Chemo Wanbang Biopharma Co. Ltd. China) in a volume of 1 mL of normal saline after CLP. Arterial blood (1 mL) was withdrawn from the inferior vena cava at 3, 6, 12, and 24 hours after surgery, and the isolated serum was stored at −70 °C for the determination of inflammatory mediators and other cardiac enzyme index. Blood loss was replaced by the same volume of hydroxyethyl starch (6% hydroxyethyl starch 130/0.6; Fresennius, Beijing, China). At the end of the 24-hour period, rats were euthanized by exsanguination with an overdose of sodium pentobarbital (60 mg/kg), the rats were perfused with 10 mL of phosphate-buffered saline (PBS) through the right ventricle to rinse the pulmonary circulation of blood. After that, hearts were removed for tissue analysis.

Enzyme-linked immunosorbent assay (ELISA)

Commercially available ELISA kits (Jiancheng Bio-Technology and Science Inc., Nanjing, China) for rats were used to measure plasma cardiac troponin I (cTnI) and brain natriuretic peptide (BNP) activities of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) according to the manufacturers' instructions. Tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-10 and C-reactive protein in the serum were measured using commercial kits (Langka Inc., Shanghai, China).

Histological analysis

After the rats were sacrificed, their cardiac left ventricular myocardium was rapidly dissected out and washed immediately with ice-cold normal saline and fixed in 10% buffered formalin. The fixed tissues were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. The sections were examined under a light microscope and photomicrographs were taken by a Zeiss Axioskop 40 photomicroscope at ×200 magnification.

Hemodynamic examination

The rats were anesthetized with chloral hydrate anesthesia (40 mg/kg i.p.). Catheters were inserted into the left ventricle through the right common carotid artery. Changes in hemodynamic parameters, including left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), the maximal rate of rise in blood pressure in the ventricular chamber (+dp/dt_{max}) and the maximal rate of decline in blood pressure in the ventricular chamber (-dp/dt_{max}) were measured and recorded by an 8-channel polygraph system (RM-6000).

Preparation of single cell suspensions

The protocol of preparation of single cell suspensions from rat heart is described as previously.[13] In brief, the rat heart tissue is thoroughly washed with an excess of ice-cold phosphate-buffered saline (PBS) without calcium and magnesium, with 200 μmol of N-1-butyl-a-phenynitrone (PBN) to remove red blood cells, then put on a 120 mesh stainless steel net. A petri dish was put under the net. Tissue is minced quickly, using a pair of sharp scissors, to approximately 1-mm^3 pieces. The tissue was gently rubbed with small surgical forceps and washed with normal saline. After that, the suspension in the petri dish was filtered with a 300 mesh copper net to remove tissue mass. The suspension was collected and centrifuged at 800 r/min for 2 minutes at room temperature. The sediment was the single cell suspension of rat heart. The supernatant fluid was removed thoroughly to exclude PBS. The concentration was adjusted to 1×10^6/mL.

Flow cytometry analysis

Cardiac myocyte apoptosis was measured by flow cytometry. Annexin V was labeled as described previously.[14] Briefly, cardiomyocytes were isolated for flow cytometry, then cell apoptosis with Annexin V-FITC/PI Apoptosis Detection Kit (BD Pharmagen, USA) was detected according to the manufacturer's protocol. Cells were harvested and adjusted to a confluency of 0.5×10^6 to 1×10^6 cells/mL. Subsequently, the cells were fixed with 70% pre-chilled alcohol and stained with propidium iodide (PI). PI-labeled cells were analyzed using flow cytometry.

Mitochondrial preparation

Sucrose gradient centrifugation was used.[15] After the rats were killed, the ventricles were excised rapidly and placed in medium (250 mmol/L sucrose, 10 mmol/L Tris-HCl buffer, pH 7.4 at 25 °C). The ventricle was cut with a pair of scissors, homogenized on ice and centrifuged at 600 g for 10 minutes. The supernatant was centrifuged for 10 minutes at 15 000 g to obtain the mitochondrial pellet. The latter was washed with the same medium and centrifuged at 15 000 g for 10 minutes. The resulting mitochondrial pellet was washed again with the same medium to get a mitochondrial suspension. The protein content was determined by the method of Lowry. Then the mitochondrial suspension was stored on ice.

Monitoring of myocardium mitochondrial inner membrane potential

1×10^6/mL 0.1 mL of mitochondrial suspension was taken into a cuvette to examine the myocardium mitochondrial inner membrane potential. 100 μL of rhodamine 123 (Sigma,USA) was added into the cuvette. It was incubated for 30 mintues at room temperature to avoid light, then 10 mL of PBS was added to wash once. The supernatant was abandoned, but preparation was used for flow cytometry examination.

Expresssion of NF-κB (p65)

The expresssion of NF-κB (p65) was evaluated by flow cytometry analysis. 1×10^6/mL or 0.1 mL of single cell suspension was put in a cuvette. 100 μL of NF-κB p65 rabbit anti-rat monoclonal antibodies (Cell Signaling Technology, USA) was added. It was incubated for 30 mintues at room temperature to avoid light, then 10 mL of PBS was added to wash once. The supernatant was abandoned, then 100 μL of goat anti-rabbit FITC-IgG (Santa Cruz Biotechnology, Inc., USA) as the second antibody of working solution was added. It was incubated for 30 mintues at room temperature to avoid light, then 10 mL of PBS was added to wash once. The supernatant was abandoned. PBS (0.1 mL) was added and filtered with a 300 mesh copper net before examination. The primary or second antibody of baseline control and negative control were set.

Survival rate

In additional CLP and EPO groups (20 for each group), rats were not subjected to samplings and were frequently observed by a researcher blinded to the group assignment to determine 7-day survival rate.

Statistical analysis

Data were expressed as mean±SD and analyzed by one-way analysis of variance or by two-tailed t test. A P value <0.05 was considered statistically significant. Significance in survival studies was examined with the log-rank test.

RESULTS

Rat sepsis model confirmation

While animals subjected to CLP appear healthy in the initial phase after the procedure, they begin to show clinical signs of sepsis at around 12 hours after surgery, including malaise, fever, chills, piloerection, generalized weakness and reduced gross motor activity. Lethality begins at 18–24 hours after surgery, indicating that the surgery under the conditions described above represents a rapidly lethal model of acute sepsis.

Hemodynamic examination

LVSP, +dp/dt_{max} and -dp/dt_{max} dereased, whereas LVEDP increased in the CLP group compared with the sham group (Table 1). Hemodynamic index significantly improved at different time in the EPO group compared with the CLP group.

Inflammatory index

CRP, TNF-α, IL-6, and IL-10 significantly increased (P<0.05, P<0.01) in the CLP group compared with the sham group (Table 2). CRP, TNF-α, and IL-6 significantly decreased in the EPO group compared with the CLP group (P<0.05), whereas IL-10 was higher at all times in the EPO group than in the CLP group (P<0.05).

Myocardial enzyme index

cTnI value peaked at 12 hours after surgery in the CLP group, 6100 times higher than that in the sham group, and significantly higher than that in the sham group and the EPO group (P<0.05) (Table 3). The cTnI value was lower in the EPO group than in the CLP group at 6, 12, 24 hours after surgery (P<0.05). CK, AST and LDH were higher to varying degrees in the CLP group than in the sham group (P<0.05). Furthermore, they were

Table 1. Effects of cecal ligature and puncture on myocardial function using the conductance catheter

Parameters	3 h	6 h	12 h	24 h
LVSP (mmHg)				
Sham	176.3±4.5	178.5±3.9	175.4±4.0	177.5±4.9
CLP	102.1±5.3*	67.4±6.1$^{\#}$	85.6±8.9$^{\#}$	92.7±7.6$^{\#}$
EPO	132.2±5.0$^{*\blacktriangle}$	119.1±5.1$^{*\blacktriangledown}$	135.4±6.7$^{*\blacktriangledown}$	138.3±5.9$^{*\blacktriangledown}$
LVEDP (mmHg)				
Sham	4.12±0.45	3.98±0.32	4.01±0.47	4.16±0.28
CLP	4.52±0.73	6.93±0.98$^{\#}$	7.03±1.21$^{\#}$	7.14±1.23$^{\#}$
EPO	4.63±0.82	6.49±1.04$^{\#}$	5.32±0.92$^{\#\blacktriangledown}$	4.98±0.86$^{\#\blacktriangledown}$
+dp/dt_{max} (mmHg)				
Sham	6840±121.7	6884±127.5	6900±122.3	6850±121.7
CLP	4002±375.7$^{\#}$	2821±372.6$^{\#}$	4366±473.8$^{\#}$	4496±504.1$^{\#}$
EPO	5471±180.3$^{\#\blacktriangledown}$	5277±153.2$^{\#\blacktriangledown}$	5350±211. 6$^{\#\blacktriangledown}$	5379±143.9$^{\#\blacktriangledown}$
-dp/dt_{max} (mmHg)				
Sham	–5737±184.7	–5740±178.6	–5800±180.2	–5772±170.8
CLP	–3821±187.3$^{\#}$	–1351±151.3$^{\#}$	–3081±416.1$^{\#}$	–3321±490.1$^{\#}$
EPO	–4546±112.1$^{\#\blacktriangledown}$	–4121±149.5$^{\#\blacktriangledown}$	–4214±109.2$^{\#\blacktriangledown}$	–4350.3±173.1$^{\#\blacktriangledown}$

Compared with the sham group, $^{*}P$<0.05, $^{\#}P$<0.01; compared with the CLP group, $^{\blacktriangle}P$<0.05, $^{\blacktriangledown}P$<0.01; LVEDP: left ventricular end-diastolic pressure; LVSP: left ventricular systolic pressure; +dp/dt_{max}: maximal rate of rise in blood pressure in the ventricular chamber; -dp/dt_{max}: maximal rate of declining in blood pressure in the ventricular chamber.

Table 2. Comparison of plasma inflammatory factors at different times after surgery

Parameters	3 h	6 h	12 h	24 h
CRP (mg/L)				
Sham	0.80±0.16	0.75±0.20	0.77±0.19	0.84±0.16
CLP	1.26±0.18	2.31±0.33$^{\#}$	1.89±0.29$^{\#}$	1.40±0.27
EPO	1.12±0.13	1.86±0.23$^{\#\blacktriangledown}$	1.45±0.20$^{\#\blacktriangledown}$	1.17±0.15
TNF-α (ng/L)				
Sham	12.92±0.91	13.24±0.77	12.65±0.95	13.07±0.85
CLP	63.69±6.85$^{\#}$	45.58±3.96$^{\#}$	41.46±5.04$^{\#}$	25.99±5.08$^{\#}$
EPO	53.57±5.48$^{\#\blacktriangledown}$	33.31±4.21$^{\#\blacktriangledown}$	29.44±2.68$^{\#\blacktriangledown}$	17.98±2.13$^{*\blacktriangledown}$
IL-6 (ng/L)				
Sham	68.71±11.48	62.50±13.22	69.47±10.25	70.03±10.72
CLP	1768.9±195.1$^{\#}$	958.7±141.6$^{\#}$	449.9±56.28$^{\#}$	198.05±58.10$^{\#}$
EPO	1129.5±151.3$^{\#\blacktriangledown}$	666.5±191.5$^{\#\blacktriangledown}$	309.3±65.4$^{\#\blacktriangledown}$	122.4±34.5$^{*\blacktriangledown}$
IL-10 (pg/mL)				
Sham	49.90±10.34	48.70±11.55	47.24±10.68	50.29±9.47
CLP	198.6±42.1$^{\#}$	711.3±84.9$^{\#}$	528.7±108.7$^{\#}$	334.2±44.2$^{\#}$
EPO	297.8±43.2$^{\#\blacktriangledown}$	1345.6±171.5$^{\#\blacktriangledown}$	798.8±61.4$^{\#\blacktriangledown}$	392.3±52.8$^{\#\blacktriangle}$

Compared with the sham group, $^{*}P$<0.05, $^{\#}P$<0.01; compared with the CLP group, $^{\blacktriangle}P$<0.05, $^{\blacktriangledown}P$ <0.01.

Table 3. Comparison of myocardial enzyme indicators at different times after surgery

Parameters	3 h	6 h	12 h	24 h
cTnI (ng/L)				
Sham	0.47±0.20	0.49±0.12	0.38±0.11	0.46±0.14
CLP	386.1±137.7*	1184.3±385.4*	2320.6±447.4*	1173.5±249.6*
EPO	344.2±111.9*	671.4±349.8$^{*▲}$	1134.7±247.6$^{*▼}$	878.6±184.9$^{*▲}$
CK (U/L)				
Sham	732±107.5	750±102.7	743±105.8	749±104.2
CLP	4632±638.4*	2704±415.8*	8638±1995.4*	10604±3660.1*
EPO	2580±941.6$^{*▼}$	1638±750.8$^{*▼}$	6060±1766.5$^{*▲}$	8728±1975.1*
AST (U/L)				
Sham	473±103.3	450±110.2	466±108.3	470±102.9
CLP	582±213.5	1233±429.1*	1865±349.1*	2543±598.4*
EPO	546±127.9	743±107.2$^{*▼}$	1199±217.1$^{*▼}$	2445±774.8*
LDH (U/L)				
Sham	763±96.2	780±85.7	769±80.8	774±90.2
CLP	1282±357.7$^{\#}$	1755±446.3$^{\#}$	2945±744.9*	4246±771.9*
EPO	1035±189.5$^{\#}$	1319±527.9$^{\#}$	1933±546.3$^{\#▼}$	4073±552.6*

Compared with the sham group, $^{a}P<0.05$, $^{\#}P<0.01$; compared with the CLP group, $^{▲}P<0.05$, $^{▼}P<0.01$.

Table 4. Comparison of the apoptosis rate of cardiomyocyte in rats 24 hours after surgery

Groups	Early apoptosis rate (%)	Late stage apoptosis rate (%)
Sham	0.27±0.31	2.88±0.35
CLP	8.54±1.33$^{\#}$	23.51±5.67$^{\#}$
EPO	1.81±0.79$^{*▼}$	9.02±2.32$^{\#▼}$

Compared with the sham group, $^{*}P<0.05$, $^{\#}P<0.01$; compared with the CLP group, $^{▼}P<0.01$.

Table 5. Comparison of the membrane potential of chondriosome of cardiomyocyte in rats 24 hours after surgery

Groups	Mitochondrial membrane potential
Sham	340.42±25.90
CLP	122.26±23.69$^{\#}$
EPO	236.25±24.02$^{*▼}$

Compared with the sham group, $^{*}P<0.05$, $^{\#}P<0.01$; compared with the CLP group, $^{▼}P<0.01$.

significantly higher in the CLP group than in the EPO group at different times.

Cardiomyocte apoptosis index

The early apoptosis rate and the late stage apoptosis rate of cardiomyocyte in the CLP group were higher than those in the sham group at 24 hours after surgery ($P<0.01$), but they were lower in the EPO group than in the CLP group ($P<0.01$) (Table 4).

Cardiomyocyte mitochondrial membrane potential

The fluorescent peak of rat cardiomyocyte significantly shifted to the left in the CLP group compared with the sham group, showing a significant decrease of mitochondrial membrane potential. The fluorescent peak in the EPO group in contrast to the CLP group shifted to the right, indicating a decline of mitochondrial membrane potential (Table 5, Figure 1).

Expression of NF-κB p65 in the rat myocardium

The expression of NF-κB p65 in the rat myocardium increased significantly in the CLP group compared with the sham group ($P<0.01$) but decreased significantly in the EPO group than in the CLP group ($P<0.01$) (Table 6, Figure 2).

Table 6. Comparison of expression levels of nuclear factor–κB p65 of myocardial tissue in rats 24 hours after surgery

Groups	NF-κB p65 in myocardial tissue
Sham	171.59±16.71
CLP	442.04±62.26*
EPO	276.23±28.04$^{*\#}$

Compared with the sham group, $^{*}P<0.01$; compared with the CLP group, $^{\#}P<0.01$.

Histological analysis of heart tissue under a light microscope

In the sham group, myocardial structure was clear, myocardium fiber was well arranged, stripes were clear, and the structure was normal. In the CLP group, however, serious cardiomycte edema, extensively myocardium spot hemorrhage, small necrosis focus, and a mass of inflammatory cells infiltration could be observed in the rat heart tissue; in the EPO group, the wavy arrangement of myocardial fibers and infiltration of inflammatory cells could be observed in the myocardium (Figure 3).

Survival rate

The survival rate of rats in the CLP group was 60% at day 1 and decreased to 30% at day 7. Although EPO administration improved the survival rate to 78% at day 1 and 50% at day 7, respectively, this increase

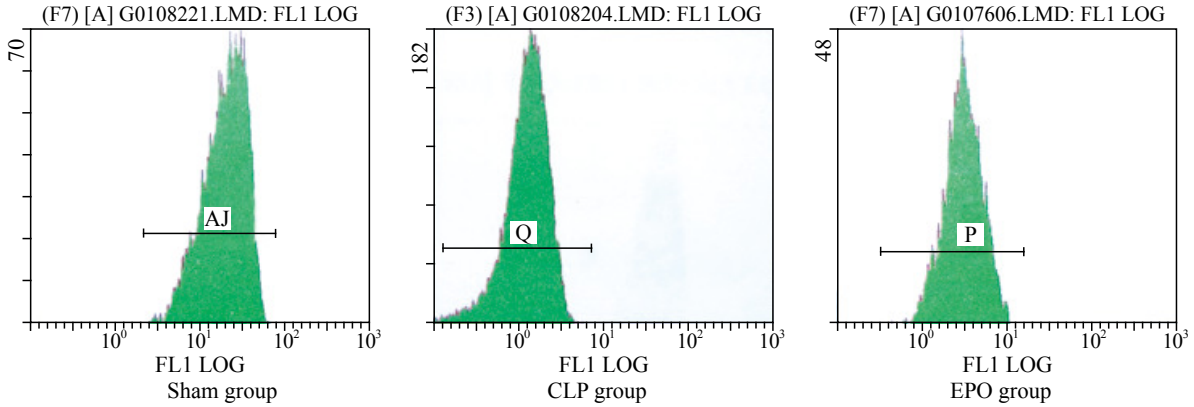

Figure 1. Comparison of membrane potential of chondriosome of cardiolmyocyte in rats 24 hours after surgery.

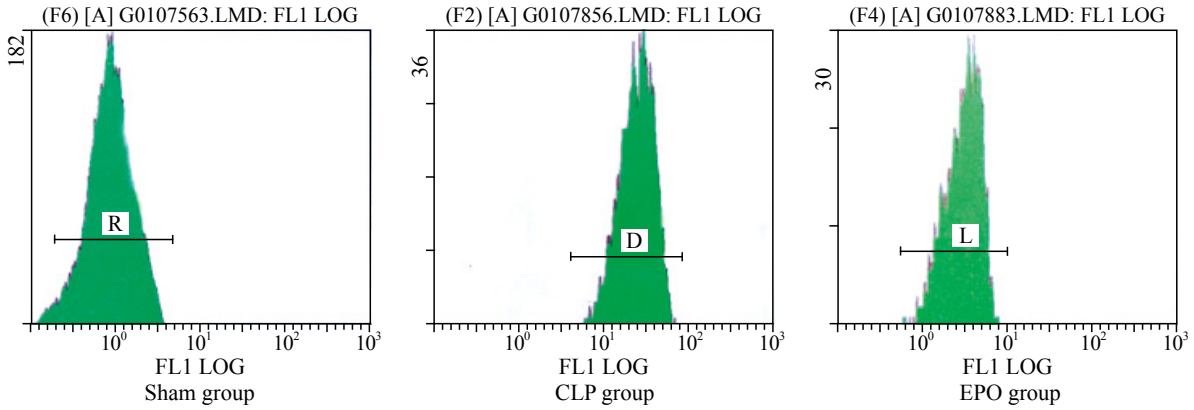

Figure 2. Comparison of expression levels of NF-κB p65 of myocardial tissue in rats 24 hours after surgery.

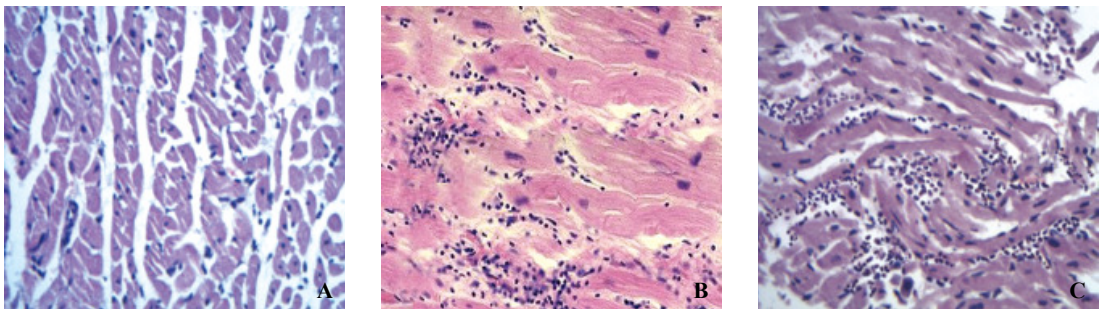

Figure 3. Pathological changes of myocardial tissue in rats 24 hours after surgery (HE×400).

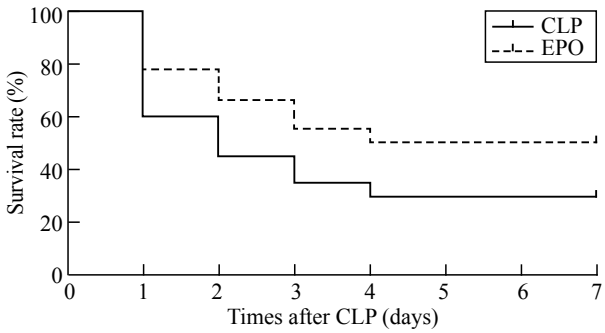

Figure 4. Survival rate at 7 days after CLP (n=20 for each group).

was not statistically significant (Figure 4, P=0.1776). The survival rates were estimated by the Kaplan-Meier method and compared by using the log-rank test. No difference in survival rates was observed between the CLP group and the EPO group.

DISCUSSION

Myocardial depression or myocardial injury is common in the severe sepsis and septic shock despite

an apparent normal or increased cardiac output. Approximately 50% of patients with hypotension due to sepsis admitted to an intensive care unit survive, and the rest 50% patients will die of refractory hypotension or multiple organ dysfunction syndromes. In 10% to 20% of these patients with refractory hypotension, there is a clinical picture of low cardiac output due to severe myocardial dysfunction.[16]

This study showed that cardiomyocyte insufficiency, sepsis-induced myocardial depression and myocardial dysfunction occurred in the rat sepsis model. This pathophysiology can be blocked by using EPO, which will improve cardiac function. LVSP and $\pm dp/dt_{max}$ are increased significantly whereas LVDP is decreased when EPO is applied. This finding indicates that EPO could improve hemodynamic status. Cardiac troponins I (cTnI) is the "gold standard" of cardiospecific markers for reflecting myocardial injury with a high sensitivity and a high specificity.[17] In our study, cTnI increased markedly in severe sepsis, peaked at 12 hours, and decreased slightly, but it was still higher than the normal value. These phenomena indicated that obvious and persistent myocardial injury occurred in severe sepsis. cTnI remarkably decreased when EPO was used, showing that EPO had a myocardium protection in the rat sepsis model. Changes of myocardial enzyme also observed in this experiment, and this indicated that myocardial enzyme decreased after the intervention of EPO. This finding suggested that myocardial injury was relieved. In addition, cardiac myocyte edema and inflammatory reaction were relieved.

Inflammatory response is a key factor for myocardial injury, and the inflammatory infectors are considered to be "myocardial depressant factors". Proinflammatory cytokines such as TNF-α, IL-6 and IL-8 have been found.[18] CRP, TNF-α, and IL-6 in serum were markedly increased in the CLP group in this study. These proinflammatory cytokines were decreased and anti-inflammatory cytokine IL-10 was up-regulated by EPO intervention. These changes indicated that EPO has marked anti-inflammatory effects.

NF-κB is a master transcription factor controlling the expression of a wide range of proinflammatory genes.[19,20] The expression of NF-κB P65 protein reflects the activation of NF-κB.[21] In the present study, the expression of NF-κB P65 protein decreased after the intervention of EPO, indicating that EPO may inhibit cytokine production through the suppression of activation of NF-κB following the relief of inflammatory injury in the myocardium. This may be one of the cardioprotective effects of EPO on sepsis-induced cardiac injury.

The abnormal apoptosis of immune cells and tissue cells in sepsis is closely related to the course and prognosis of diseases. Treatments correcting abnormal apoptosis have been developed with good therapeutic results in animal experiments.[22,23] In the present study, cell apoptosis was detected by flow cytometry. The early and late stage apoptotic rate of cardiomyocytes significantly increased in the EPO group and CLP group compared with the sham group 24 hours after occurrence of sepsis induced injury. The early apoptosis rate was significantly lower in the EPO group than in the CLP group. The results indicated that EPO suppresses cardiomyocyte apoptosis in sepsis and exogenous EPO relieves sepsis induced cardiomyocyte apoptosis.[24,25]

Normal mitochondrial transmembrane potential is a prerequisite for oxidative phosphorylation and production of aerobic adenosine triphoshate. Mitochondrial dysfunction is thought to play an important role in the pathogenesis of a wide variety of diseases, including sepsis.[26] It has been proposed that an acquired defect in oxidative phosphorylation prevents production of aerobic adenosine triphosphate and potentially causes sepsis-induced organ dysfunction.[27] The reduction of mitochondrial transmembrane potential is an irreversible event in the early stage of apoptosis.[28,29] The present study showed that the mitochondrial transmembrane potential of cardiomyocyte decreased in sepsis. Compared with the CLP group, the fluorescent peak of rat cardiomyocytes significantly shifted to the right in the EPO group, showing the cells with an increased fluorescence intensity. The results implied that EPO blocked the reduction of mitochondrial transmembrane potential and suppressed the apoptosis of cardiomyocytes.

In summary, the present study provided evidence that exogenous EPO blocked the reduction of mitochondrial transmembrane potential and suppressed cardiomyocyte apoptosis. At the same time, EPO inhibited the activation of NF-κB, reduced the production of proinflmmatory cytokines, and relieved sepsis-induced myocardium inflammatory injury. The two mechanisms play roles in the protection of sepsis-induced myocardium injury and the improvement of heart function.

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Ethical approval: The study was approved by the Animal Care and Use Committee of the Third Hospital of Hebei Medical University, Shijiazhuang, China.

Conflicts of interest: The authors declare that there is no conflict of interest.

Contributors: Qin YJ designed the research, analyzed the data, and wrote the paper. All authors read and approved the final version.

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