

## Original Article

# Glucocorticoids offer protection against myocardial injury in a murine model of sepsis

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**Abstract:** Sepsis is a serious infection-related complication that, in causing significant inflammation, often leads to myocardial injury. Severe inflammation, including in sepsis, is sometimes treated with exogenous glucocorticoids (GCs). Here, to explore the potential effect of GCs to protect against myocardial injury, we created a model of sepsis in rats by performing cecal ligation and puncture (CLP) in 96 rats randomly divided into sham-operated control (N=32), untreated sepsis (CLP, N=32), and GC-treated sepsis (N=32) groups. At 3, 6, 12, and 24 h after surgery, the changes in cardiac hemodynamic indexes, serum inflammatory response factor levels, and myocardial enzymes were measured, along with mitochondrial membrane potential in myocardial cells, apoptosis of myocardial cells, and the expression of nuclear factor kappa B (NF- $\kappa$ B p65) in myocardial tissues. Pathological changes in myocardial cells were also observed. Compared to the sham-operated group, CLP rats experienced deterioration of left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum rate of left ventricular pressure rise (+dP/dt<sub>max</sub>), and the maximum rate of left ventricular pressure drop (-dP/dt<sub>max</sub>). CLP rats also had a rise in serum tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), C-reactive protein (CRP), cardiac troponin I (cTnI), creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and NF- $\kappa$ B p65 in myocardial tissues. The GCs-treated group had lower levels of these inflammatory response molecules than the CLP group, with the exception of anti-inflammatory cytokine interleukin-10 (IL-10), which was higher in the GC-treated rats than the CLP group at each time point post-surgery. Compared to the sham group, CLP rats had a rise in myocardial cell apoptosis and a drop in mitochondrial membrane potential in myocardial cells. In addition, GCs-treated rats had a marked drop in the myocardial cell apoptosis rate and a rise in the mitochondrial membrane potential compared to CLP rats. After intervention with GCs, the pathological changes in heart tissues were also reduced compared to those in the sepsis group. Based on these results, we conclude that exogenous GCs can inhibit a drop in myocardial mitochondrial membrane potential and inhibit myocardial cell apoptosis by blocking the activation of NF- $\kappa$ B, decreasing the generation of proinflammatory cytokines, and relieving inflammatory injury in heart tissues.

**Keywords:** Myocardial injury, apoptosis, mitochondrial membrane potential, nuclear transcription factor, rat

## Introduction

Sepsis is a common, life-threatening complication that occurs following infection. The infection triggers an inflammatory response that releases many cytokines and chemokines throughout the body, which ultimately negatively affects multiple organs. Its pathogenesis is influenced by myocardial depression in 80% of severe sepsis cases. Importantly, treatment options in sepsis remain limited. Although positive inotropes can improve the ejection fraction, their benefits are not guaranteed due to increased myocardial oxygen consumption [1].

Thus, there are no approaches to effectively improve myocardial depression.

Glucocorticoids (GCs) are commonly used to treat severe inflammatory diseases, including sepsis. GCs activate the glucocorticoid receptor pathway to enhance the activity of histone deacetylase 2 and inhibit the transcription of proinflammatory nuclear factor kappa B (NF- $\kappa$ B) and activator protein-1 (AP-1) [2]. The effects of GCs on myocardia have come to the forefront in recent years; GCs can directly act on myocardial cells and have protective effects on the cardiovascular system [3, 4]. Recent evidence indi-

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cates that GCs have protective effects against sepsis in animals, but there are no reports on the effects against myocardial depression or myocardial injury in sepsis. We sought to construct models of myocardial injury in rats with sepsis and perform intervention with GCs, thereby exploring the protective effects of GCs against myocardial injury in sepsis to provide a theoretical basis for clinical treatment.

### Materials and methods

#### *Animal models of sepsis*

Ninety-six healthy male Sprague-Dawley rats (210-250 g) were provided by the Laboratory Animal Center of Nanjing Medical University (Nanjing, Jiangsu Province, China) and raised with free access to food and water. Rats were randomly assigned to three groups using a random number table: an untreated sepsis group (CLP group, N=32), a treated sepsis group (GCs-treated group, N=32), and a sham-operated control group (sham group, N=32). Cecal ligation and puncture (CLP) were performed to induce sepsis according to an established protocol [5]. 10% chloral hydrate (0.03 mL/kg) (Tianjin Kemiou Chemical Reagent Co., Ltd., Tianjin, China) was subcutaneously injected as an anesthetic, and rats were fixed and covered under aseptic hole-towels. An ~1.5-cm incision was made along the midline of the abdomen to expose the cecum. The cecum was ligated approximately 1.5 cm from its root; ligation within the blood vessels of the ileum and meso-ecum was avoided. An 18-gauge needle was used to puncture the cecum 3 times and then a rubber drainage tube was inserted to prevent puncture site closure. The cecum was squeezed to allow stools to overflow and form a leak. Then the cecum was reinserted in the abdominal cavity, and the abdominal wall incision was sutured. GCs-treated rats received Hydrocortisone (10 mg/Kg, The Ninth Pharmaceutical Factory of Shanghai) immediately after surgery via intraperitoneal injection. The sham group underwent a sham surgery in which the abdominal cavity was opened to find the cecum and the abdominal wall incision was sutured, but no puncturing was done.

#### *Identification of sepsis*

After surgery, the experimental animals regained consciousness very quickly. Rats with sepsis presented with listlessness, lethargy, delayed response, inactivity, fear of cold, hud-

dling together, piloerection, anorexia, and increased secretions at the canthi. CLP rats displayed a large amount of purulent exudate visible to the naked eye on the small intestine serosal surface. Varying degrees of inflammatory cell infiltration were observed in the tissues: inside the alveolar space, there were inflammatory exudates, peeling alveolar epithelia, as well as focal necrosis of liver cells, which was accompanied by inflammatory cell infiltration. The control group showed far fewer or no abnormalities in organ secretion.

#### *Cardiac hemodynamic indexes*

After induction of sepsis, rats were subcutaneously injected with 10% chloral hydrate (0.03 mL/kg) as an anesthetic. Then, the right common carotid artery was separated and a catheter was inserted so that a polygraph could be used to measure hemodynamic parameters: left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum rate of left ventricular pressure rise (+dP/dt<sub>max</sub>), and the maximum rate of left ventricular pressure drop (-dP/dt<sub>max</sub>).

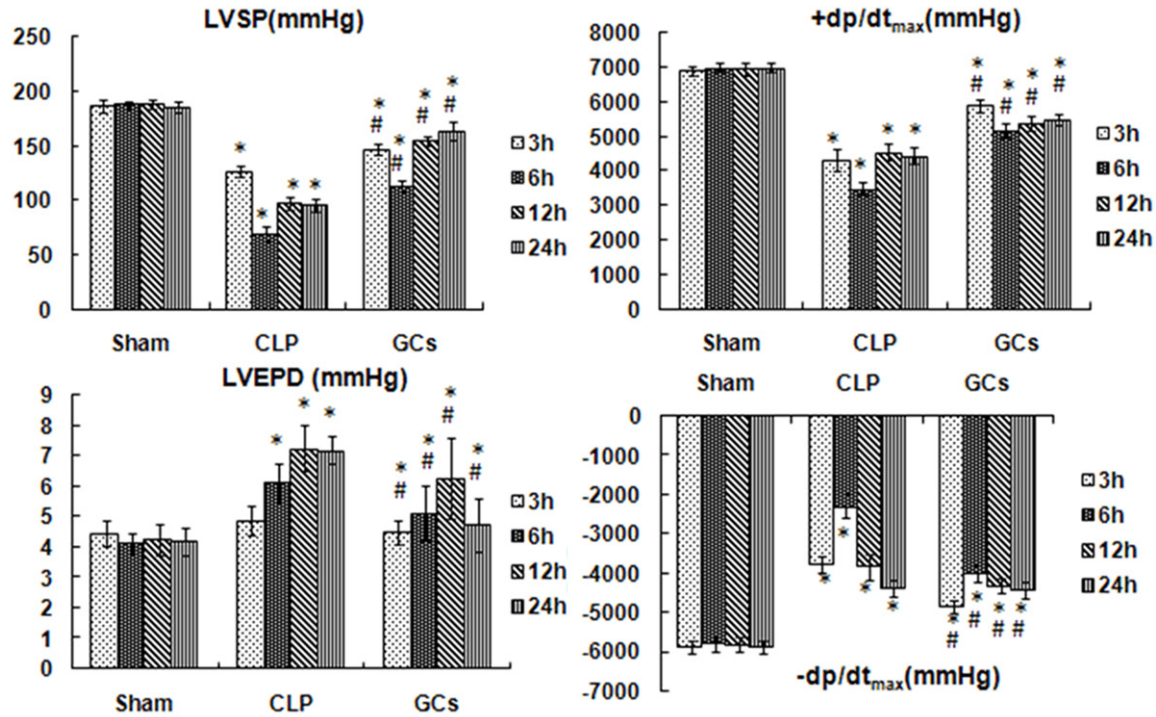
#### *Preparation and storage of rat sera*

5 mL of blood were taken from the inferior vena cava and 2.5 mL of blood were placed in a tube containing heparin as an anticoagulant. After mixing, the tube was placed in a refrigerator at 4°C until ready for use in the detection of cardiac troponin I (cTNI) and B-type natriuretic peptide (BNP) by chemiluminescence method. The remaining blood was placed at room temperature for 2 h, then centrifuged to collect serum. The serum was stored at -74°C until use in the detection of serum tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), interleukin 10 (IL-10), C-reactive protein (CRP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK). Rat IL-6, IL-10, and TNF-α were detected using the appropriate ELISA kits purchased from the Shanghai Langka Company (Shanghai, China). Rat cTNI, LDH, AST, CK, and CRP ELISA kits were provided by the Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and utilized according to the manufacturer's instructions.

#### *Preparation of single-cell suspension*

Fresh myocardial tissues were placed on a 120-mesh stainless steel net with a plate underneath. The tissues were trimmed with

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**Figure 1.** Comparison of cardiac hemodynamic at different time in rats after the surgery ( $n=32$ ). Note: \*:  $P<0.05$  vs. Sham group, #:  $P<0.05$  vs. CLP group.

ophthalmic scissors and gently rubbed with ophthalmic forceps while being rinsed with normal saline. The suspension of cells that collected in the plate was then passed through a 300-mesh copper net to remove cell aggregates. The cell suspension was centrifuged at 500-800 rpm for 2 min and the precipitate was collected as a single-cell suspension. The supernatant was removed and the concentration was adjusted to  $1 \times 10^6/\text{mL}$ .

### Detection of myocardial apoptosis

100  $\mu\text{L}$  of the cell suspension was placed in a 5 mL flow tube. Then, 5  $\mu\text{L}$  of Annexin V/FITC and 10  $\mu\text{L}$  of propidium iodide (20  $\mu\text{g}/\text{mL}$ ) (Pharmagen, Silver Spring, MD) were added. After shaking, the mixture was allowed to undergo a reaction at room temperature away from light for 15 min. 500  $\mu\text{L}$  of Buffer was added to the tube for flow cytometric analysis using a FACS Calibur low laser cytometer (Becton Dickinson, USA).

### Detection of mitochondrial membrane potential

0.1 mL of  $1 \times 10^6/\text{mL}$  single-cell suspension was added to 100  $\mu\text{L}$  of Rhodamine 123 (Sigma-

Aldrich, St. Louis, MO) and incubated at room temperature away from light for 30 min. Next, 10 mL of PBS were used to wash the cells once before the supernatant was discarded and the remaining fluid was used for flow cytometric analysis.

### Detection of NF- $\kappa\text{B}$ p65 in myocardia

0.1 mL of the  $1 \times 10^6/\text{mL}$  single-cell suspension was added to 100  $\mu\text{L}$  of NF- $\kappa\text{B}$  p65 rabbit anti-rat monoclonal antibody (Cell Signaling Technology, Danver, MA) and incubated at room temperature away from light for 30 min. 10 mL of PBS were used to wash once, and then the supernatant was discarded before 100  $\mu\text{L}$  of goat anti-rabbit FITC-IgG (SantaCruz Biotechnology, Dallas, Texas) were added as the secondary antibody. The mixture was incubated at room temperature away from light for 30 min and then added to 10 mL of PBS and centrifuged. Finally, the supernatant was discarded to remove the nonconjugated fluorescent secondary antibody, and the remaining solution was added to 0.1 mL of PBS and passed through a 500-mesh copper net before detection in an Epics-XL II flow cytometer (Beckman Coulter, Brea, CA). When immunofluorescence-

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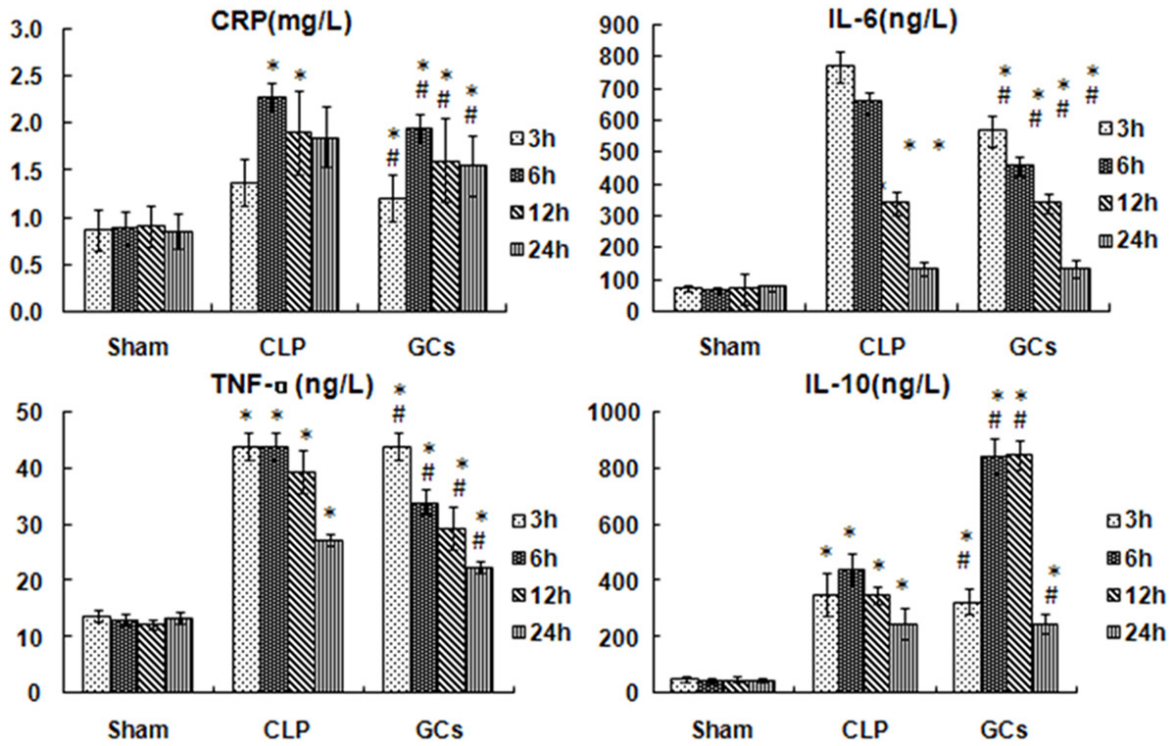


Figure 2. Serum indexes of inflammatory response after sepsis modeling in rats (n=32). Note: \*: P<0.05 vs. Sham group, #: P<0.05 vs. CLP group.

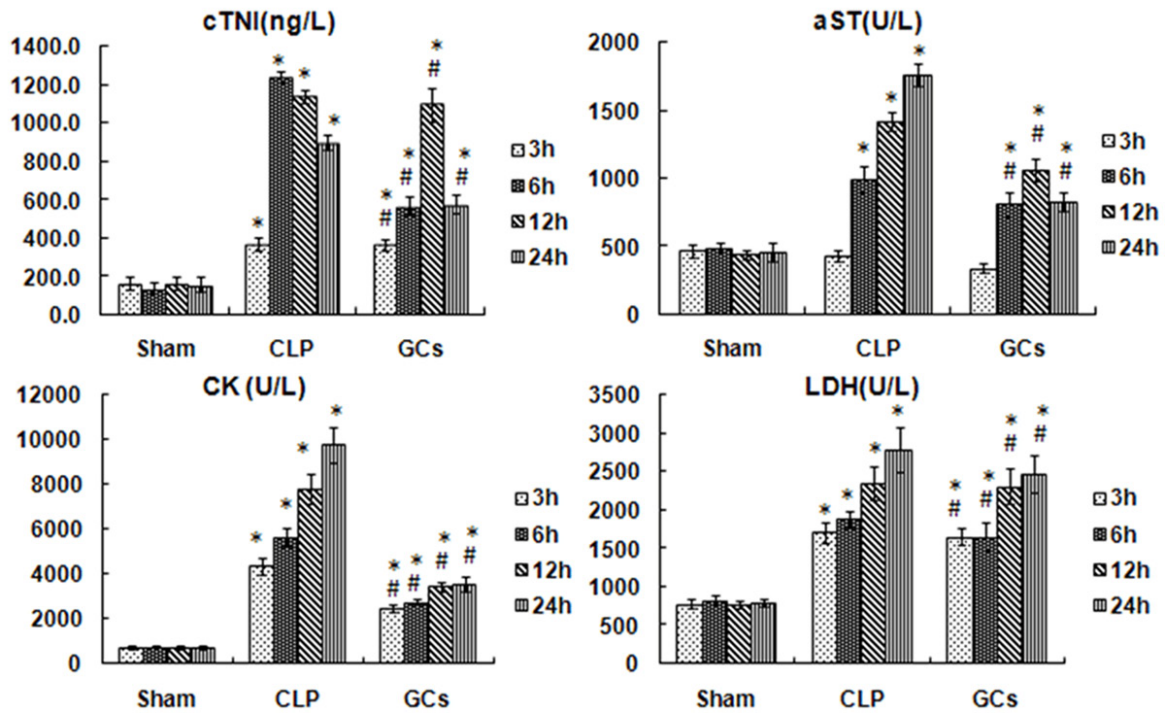
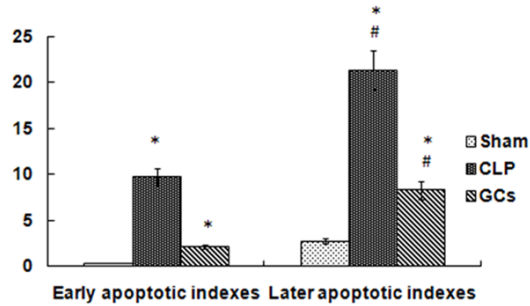
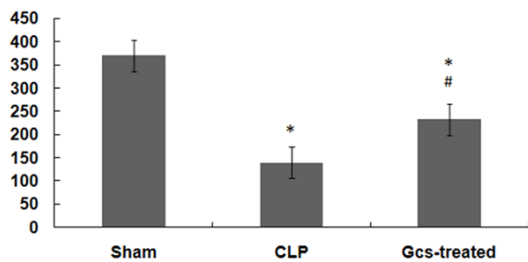


Figure 3. Serum myocardial enzyme indexes at after sepsis modeling in rats (n=32). Note: \*: P<0.05 vs. Sham group, #: P<0.05 vs. CLP group.

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**Figure 4.** Myocardial apoptotic indexes 24 h after sepsis modeling in rats (%;  $n=32$ ). Note: \*:  $P<0.05$  vs. Sham group, #:  $P<0.05$  vs. CLP group.



**Figure 5.** Mitochondrial membrane potential in myocardial cells 24 h after sepsis modeling in rats ( $n=32$ ). Note: \*:  $P<0.05$  vs. Sham group, #:  $P<0.05$  vs. CLP group.

labelled marker proteins were identified, a background control and negative control were designed by adding the primary antibody or secondary antibody.

### Heart tissue section generation

Routine HE-stained paraffin sections were generated and observed under the light microscope (Olympus CX22).

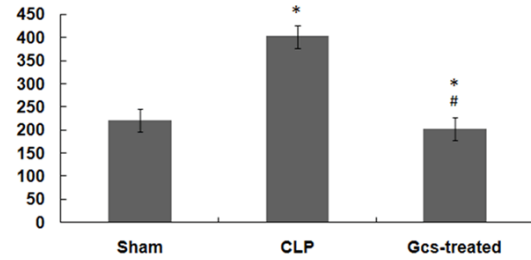
### Statistical analysis

SPSS 17.0 (IBM, Armonk, NY) was used to perform data analysis. Measurement data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm S$ ) and the differences among groups were analyzed using the t-test or one-way analysis of variance (ANOVA). A  $P<0.05$  was considered to be statistically significant.

## Results

### Generation of sepsis in experimental animals

After surgery, all experimental animals regained consciousness quickly and cleaned them-



**Figure 6.** NF- $\kappa$ B p65 expression levels in heart tissues 24 h after sepsis modeling in rats ( $n=32$ ). Note: \*:  $P<0.05$  vs. Sham group, #:  $P<0.05$  vs. CLP group.

selves. Both the CLP group and the GC-treated rats presented with significantly accelerated heart rates and breathing, poor mental status, lethargy, markedly rising temperature, anorexia, piloerection, diarrhea, as well as hemorrhage and/or hemorrhagic tendency. Approximately 3 h after surgery, the sham group returned to normal activity and food and water intake. The 7-day survival rates of the sham group, CLP group, and GCs-treated groups were 70%, 30%, and 50%, respectively.

### Cardiac hemodynamic indexes are improved in GC-treated rats

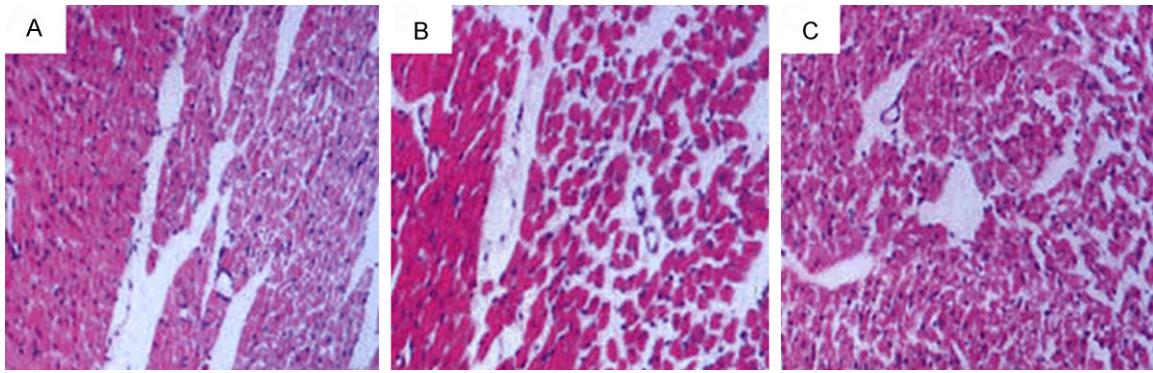
Compared to the sham group, CLP rats presented with a significant rise ( $P<0.05$ ) in VSP,  $+dp/dt_{max}$ , and LVEDP as soon as 3 h after surgery (**Figure 1**). The GCs-treated group had a significant improvement ( $P<0.05$ ) compared to the CLP group in all hemodynamic indexes at the different time points.

### Inflammatory response factors are altered in response to GC treatment

Compared to the sham group, the CLP group had a marked rise in the CRP, TNF- $\alpha$ , IL-6, and IL-10 ( $P<0.05$ ,  $P<0.01$ , **Figure 2**). Compared to the CLP group, the GC-treated group had a drop in the levels of CRP, TNF- $\alpha$ , and IL-6 ( $P<0.05$ ) and higher IL-10 levels ( $P<0.05$ ) at the different time points after surgery.

### Myocardial enzyme indexes are elevated in CLP rats

CLP rats exhibited cTNI levels reaching their peak 12 h after modeling, which was markedly elevated compared to the sham group (**Figure 3**). The cTNI levels at the various time points were significantly higher than those in the sham group ( $P<0.05$ ). Compared to the sham group,



**Figure 7.** Pathological changes of myocardium in rats 24 h after the surgery (HE  $\times 200$ ). (A) Sham group (B) CLP group (C) GCs group.

the CLP group had increased CK, AST, and LDH ( $P < 0.05$ ). In addition, the levels of CK, AST, and LDH at the various time points were higher than those in the GCs-treated group ( $P < 0.05$ ).

*Myocardial apoptosis is rescued in GC-treated rats*

Compared to the sham group, myocardia from the CLP group had a rise in both early and late apoptotic cells 24 h after modeling ( $P < 0.01$ , **Figure 4**). Both early and late apoptosis was observed in the GCs-treated group to be lower than that in the CLP group ( $P < 0.01$ ).

*Mitochondrial membrane potentials are improved in GC-treated rats*

Compared to the sham group, the CLP group had a markedly left-shifted fluorescence peak in myocardial cells, indicating that the mitochondrial membrane potential had dropped markedly ( $P < 0.01$ , **Figure 5**). Compared to the CLP group, GCs-treated rats had a markedly right-shifted fluorescence peak, indicating that the drop in the mitochondrial membrane potential was improved ( $P < 0.01$ ).

*NF- $\kappa$ B p65 expression levels are reduced in GC-treated rat heart cells*

Compared to the sham group, CLP rats had markedly elevated NF- $\kappa$ B p65 expression levels 24 h after modeling ( $P < 0.01$ ). Compared to the CLP group, GCs-treated rats had a significantly reduced NF- $\kappa$ B p65 expression level ( $P < 0.01$ , **Figure 6**).

*GC-treatment altered myocardial structures*

Morphological changes in rat hearts were observed under a light microscope (200 $\times$  mag-

nification, **Figure 7**). In the sham group, clear myocardial structures were visible and regularly arranged cardiac muscle fibers were observed with clear cross striations and normal structures. In the CLP group, significant myocardial cellular edemas were visible, small focal hemorrhages and necroses appeared widely in the myocardia, and there was a large amount of inflammatory cell infiltration in the myocardia. In the GC-treated group, cardiac muscle fibers appeared to be arranged undulantly, and there was inflammatory cell infiltration in the myocardia.

**Discussion**

During the onset of sepsis, bacteria and their products stimulate neutrophils, monocytes, macrophages, and endothelial cells to produce and release a great number of cytokines, which induce myocardial apoptosis. Myocardial apoptosis reduces the number of myocardial cells and leads to the deterioration of cardiac function, a hallmark of septic shock [6]. Sepsis presents with myocardial injury and myocardial depression, and when it becomes severe, 40%-50% of patients experience cardiac insufficiency [7]. Our study showed that a rat model of sepsis experienced severe myocardial injury, cardiac function decline, and abnormal myocardial cell function. However, this pathology was significantly improved by glucocorticoid treatment.

GCs-treated rats had elevated LVSP, reduced LVDP, and elevated  $\pm dp/dt_{max}$ , suggesting that GCs played a role in the improvement of hemodynamic performance. cTNI is only found in myocardial cells, and a rise in its level is a highly sensitive marker for myocardial injury.

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Therefore, serum cTNI levels can indirectly reflect the severity of myocardial cell injury [8]. We found that under septic conditions, cTNI levels rose significantly, reaching a peak at 12 h and remaining significantly higher than normal values, implicating persistent myocardial injury. Following the intervention with GCs, cTNI levels dropped significantly, suggesting that GCs played a protective role against myocardial injury. Observation under the light microscope showed marked changes with GC treatment, signifying that GC intervention could markedly reduce myocardial enzymes, relieve myocardial cellular edema, and improve muscle fiber structure and inflammatory response.

GCs are known to down-regulate proinflammatory cytokines, including TNF- $\alpha$ , IL-6, and IL-8 [9-13]. In this study, CLP rats had a marked rise in serum CRP, TNF- $\alpha$ , and IL-6, while the GC-treated group had a marked drop in these levels and marked up-regulation of IL-10, an anti-inflammatory cytokine, suggesting that GCs play a significant role against the inflammatory response. NF- $\kappa$ B is considered to be a genetic switch for inflammatory responses and a regulatory cytokine at the core in the inflammatory signaling pathway. Exogenous GCs with broad-spectrum anti-inflammatory effects are widely used to treat multiple inflammations and autoimmune diseases [11]. NF- $\kappa$ B P65 protein levels were reduced after intervention with GCs, suggesting that GCs may inhibit NF- $\kappa$ B activation and reduce the expression of its downstream inflammatory mediators. This may be one of the important mechanisms through which GCs protect myocardia.

When sepsis occurs, there is abnormal apoptosis in immune and tissue cells that is closely correlated with the duration and prognosis of the disease. Therapeutic measures for correcting abnormal apoptosis have been developed gradually and have shown promising results in animal models [14-18]. Our study utilized flow cytometry to detect apoptosis and found that compared to the sham group, the GC-treated and CLP groups had marked increases in both early and late heart cell apoptotic rates 24 h after septic injury. The early apoptotic rate in the GC-treated group was significantly lower than that in the CLP group, suggesting that GCs can inhibit myocardial apoptosis after sepsis.

Mitochondrial transmembrane potential is necessary for maintaining normal mitochondrial

oxidative phosphorylation and adenosine triphosphate production necessary for mitochondrial function [19]. A drop in the mitochondrial transmembrane potential is an irreversible event in the early stages of apoptosis. Our study confirmed that when sepsis occurred, the mitochondrial function of organ cells changed, and the mitochondrial membrane potential dropped. Compared to the CLP group, the GC-treated cardiac cells had a markedly right-shifted fluorescence peak, indicating that the drop in mitochondrial membrane potential seen in the CLP cells was inhibited, blocking the progression of apoptosis.

In summary, our experiments demonstrate that exogenous GCs given to rat models of sepsis can inhibit the ill-fated drop in myocardial mitochondrial membrane potential associated with apoptosis and inhibit the activation of NF- $\kappa$ B, decrease the generation of proinflammatory cytokines, and relieve inflammatory injury in heart tissues. These mechanisms play protective roles in the injured myocardia and improve heart function.

### Disclosure of conflict of interest

None.

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### References

- [1] Arai K, Lee K, Berthiaume F, Tompkins RG, Yarmush ML. Intrahepatic amino acid and glucose metabolism in a D-galactosamine-induced rat liver failure model. *Hepatology* 2001; 34: 360-371.
- [2] Bitton A, Buie D, Enns R, Feagan BG, Jones JL, Marshall JK, Whittaker S, Griffiths AM, Panaccione R. Canadian Association of Gastroenterology Severe Ulcerative Colitis Consensus Group. Treatment of Hospitalized Adult Patients With Severe Ulcerative Colitis: Toronto Consensus Statements. *Am J Gastroenterol* 2012; 107: 179-194.
- [3] Sakai H, Park SS, Kikkawa Y. Differential oxidase activity of hepatic and pulmonary microsomal cytochrome P-450 isozymes after treatment with cytochrome P-450 inducers. *Biochem Biophys Res Commun* 1992; 187: 1262-1269.

## GCs against myocardial injury in sepsis

- [4] Cheifetz AS, Stern J, Garud S, Goldstein E, Malter L, Moss AC, Present DH. Cyclosporine is safe and effective in patients with severe ulcerative colitis. *J Clin Gastroenterol* 2011; 45: 107-112.
- [5] Rahman TM, Selden AC, Hodgson HJ. A novel model of acetaminophen-induced acute hepatic failure in rabbits. *J Surg Res* 2002; 106: 264-272.
- [6] Ng SC, Chan FK, Sung JJ. Review article: the role of non-biological drugs in refractory inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; 33: 417-427.
- [7] Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; 353: 2462-2476.
- [8] Kalpana K, Ong HS, Soo KC, Tan SY, Prema Raj J. An improved model of galactosamine-induced fulminant hepatic failure in the pig. *J Surg Res* 1999; 82: 121-130.
- [9] Frühauf NR, Oldhafer KJ, Westermann S, Sotiropoulos GC, Kaiser GM. Acute hepatic failure in swine: hepatectomy versus vascular occlusion. *J Invest Surg* 2004; 17: 163-171.
- [10] Tuñón MJ, Alvarez M, Culebras JM, González-Gallego J. An overview of animal models for investigating the pathogenesis and therapeutic strategies in acute hepatic failure. *World J Gastroenterol* 2009; 15: 3086-3098.
- [11] Lee WM. Etiologies of acute liver failure. *Semin Liver Dis* 2008; 28: 142-152.
- [12] Gregus Z, Madhu C, Klaassen CD. Species variation in toxication and detoxication of acetaminophen in vivo: a comparative study of biliary and urinary excretion of acetaminophen metabolites. *J Pharmacol Exp Ther* 1988; 244: 91-99.
- [13] Doering CB, Parker ET, Nichols CE, Lollar P. Decreased factor VIII levels during acetaminophen-induced murine fulminant hepatic failure. *Blood* 2003; 102: 1743-1744.
- [14] Mowat C, Cole A, Windsor A, Ahmad T, Arnott I, Driscoll R, Mitton S, Orchard T, Rutter M, Younge L, Lees C, Ho GT, Satsangi J, Bloom S. IBD Section of the British Society of Gastroenterology. Guidelines for the management of inflammatory bowel disease in adults. *Gut* 2011; 60: 571-607.
- [15] Miller DJ, Hickman R, Fratter R, Terblanche J, Saunders SJ. An animal model of fulminant hepatic failure: a feasibility study. *Gastroenterology* 1976; 71: 109-113.
- [16] Francavilla A, Makowka L, Polimeno L, Barone M, Demetris J, Prelich J, Van Thiel DH, Starzl TE. A dog model for acetaminophen-induced fulminant hepatic failure. *Gastroenterology* 1989; 96: 470-478.
- [17] D'Haens G, Lemmens L, Geboes K, Vandeputte L, Van Acker F, Mortelmans L, Peeters M, Vermeire S, Penninckx F, Nevens F, Hiele M, Rutgeerts P. Intravenous cyclosporine versus intravenous corticosteroids as single therapy for severe attacks of ulcerative colitis. *Gastroenterology* 2001; 120: 1323-1329.
- [18] Ladurner R, Hochleitner B, Schneeberger S, Barnas U, Krismer A, Kleinsasser A, Offner F, Königsrainer I, Margreiter R, Königsrainer A. Extended liver resection and hepatic ischemia in pigs: a new, potentially reversible model to induce acute liver failure and study artificial liver support systems. *Eur Surg Res* 2005; 37: 365-369.
- [19] Ogata H, Matsui T, Nakamura M, Iida M, Takazoe M, Suzuki Y, Hibi T. A randomised dose finding study of oral tacrolimus (FK506) therapy in refractory ulcerative colitis. *Gut* 2006; 55: 1255-1262.