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Original Contribution

Esmolol reduces apoptosis and inflammation in early sepsis rats with abdominal infection

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ABSTRACT

Background: Esmolol is a highly selective beta 1 receptor blocker with various effects such as slowing heart rate, lowering blood pressure and reducing myocardial oxygen consumption. However, few studies have reported the use of beta blockers in sepsis with multiple organ dysfunctions. This study aimed to investigate the effects of esmolol on reducing apoptosis and inflammation in early sepsis rats with abdominal infection.
Methods: Rats were randomly divided into sham operation group, sepsis group, antibiotic group, Esmolol + antibiotic group with low, median and high dose Esmolol (L group, M group and H group). Values between two or more groups were compared by independent *t*-tests.
Results: In the liver and kidney, we found inflammatory infiltration in sepsis group while pathological aspects reduced in L, M and H groups. Bcl-2 mRNA and protein levels increased while Bax mRNA and protein levels decreased in the liver and kidney of L, M and H groups. Serum IL-6, HMGB-1 and TNF-α levels decreased but IL-10 level increased in L, M and H groups, compared to sepsis group. Compared to sepsis and antibiotic groups, the levels of myocardial enzymes were lower in L, M and H groups.
Conclusion: The administration of esmolol in early sepsis may reduce inflammation, inhibit apoptosis and protect key organs.

1. Introduction

Sepsis is a systemic inflammatory response caused by infection and can lead to multiple organ dysfunction syndrome (MODS) [1]. Severe sepsis induced by abdominal infection can develop rapidly with high mortality. Among abdominal organs, dysfunctions of the liver and kidney are commonly associated with abdominal infection and severe sepsis [2]. In sepsis, the body can produce inflammatory factors, resulting in systemic inflammatory response that causes damage to abdominal organs as well as myocardial injury. Interleukin (IL-6, IL-10) and high mobility group protein B1 (HMGB-1) have been shown to play important role in the development of sepsis [3]. Current therapies for septic shock mainly include sepsis bundle such as early hemodynamics assessment, volume resuscitation, early application of broad spectrum antibiotics and short-acting hormone, and blood purification [4]. However, there is no effective means to inhibit the inflammation or sequential multiple organ dysfunction caused by sepsis. It is also unclear whether highly selective short-acting beta-1 blocking agents can block sympathetic activation and thus alleviate damage to systemic organs. Since beta-blockers may aggravate cardiovascular dysfunction and low blood pressure as well as other diseases, they have not yet been generally used in patients with sepsis shock [5,6].

This study aimed to identify new agent that could relieve the inflammation and damage to vital organs of the body associated with sepsis. Esmolol is a highly selective beta 1 receptor blocker with various effects such as slowing heart rate, lowering blood pressure and reducing myocardial oxygen consumption. We established a model of sepsis in rats and investigated whether esmolol can alleviate the combined organ dysfunction caused by sepsis.

2. Materials and methods

2.1. Animals

A total of 55 male clean SD rats (weight 250–350 g) were purchased from the laboratory animal center of college of Life Sciences, Nankai University. The rats were randomly divided into a sham-operated

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control group (con group, 5 rats), sepsis group (CLP group,10 rats), antibiotic group(antibiotic group, 10 rats)and esmolol + antibiotic group, which was further divided into low dosage (L group), medium dosage (M group) and high dosage (H group) esmolol groups with 10 rats in each group. Esmolol and piperacillin/tazobactam were kindly provided by the Intensive Care Unit of Tianjin Medical University Cancer Institute and Hospital.

2.2. Establishment of animal model

Cecal ligation and puncture (CLP) method was used to model sepsis in rats [7]. Briefly, 10% chloral hydrate was injected at 0.6 ml/100 g for anesthesia and the rats were fixed, abdominal hair was removed for disinfection. Then 1.5 cm incision was made at the midline of abdomen, find caecum, peel off mesentery using No. 4 surgical suture for ligation at the point 1/3 to the bottom of caecum, and the ligation of ileum and mesenteric vascular was avoided. Next, one-time puncture of caecum was made at 0.8–1.0 cm from ligation to far end, and the ligation of bilateral detachment of omentum majus. Finally, abdominal incision was sutured and rats received immediate subcutaneous injection of 3–5 ml normal saline to prevent shock.

In Control group, rats were operated without cecal ligation and puncture. In CLP group, rats received no drug after CLP operation. In Antibiotic group, rats received intravenous administration of 787.5 mg/Kg piperacillin/tazobactam one hour after CLP operation. In Esmolol + antibiotic group, the rats received intravenous administration of 787.5 mg/Kg piperacillin/tazobactam along with 1.75 mg/Kg/h esmolol (Low dosage Group), 2.6 mg/Kg/h esmolol (Medium dosage Group), and 3.5 mg/Kg/h esmolol (High dosage Group) one hour after CLP operation till the end of the experiment.

2.2.1. Blood collection and analysis

4 ml blood was collected from the rats in each group 6 h and 24 h after the surgery. Half (2 mL) of each sample was added to an anticoagulation tube with heparin sodium, mixed and kept at 4 °C for cardiac troponin (cTnI), creatine kinase (CK) and lactate dehydrogenase (LDH) tests. The remaining 2 ml of each sample was kept at room temperature for 2 h, centrifuged to obtain serum and kept at −80 °C. Serum levels of IL-6, IL-10, HMGB-1 and TNF-α were detected using Enzyme-linked immunosorbent assay (ELISA) kits (Abcam, USA).

2.3. Analysis of the organs

All the rats were killed and the heart, livers, kidneys and lungs were dissected. Part of the organs was fixed in 4% formalin for hematoxylin and eosin (HE) staining and pathological analysis. The other part of the organs was lysed for Real-time PCR and Western blot analysis of Bcl-2 and Bax expression levels. Bcl-2, Bax and β-actin antibodies were purchased from Sigma (St Louis, MO, USA). PCR primers and SYBR GREEN kit were purchased from Takara Bio Inc. Primer sequences were as follow: Bcl-2 GGGAGATCGTGATGAAGTAC and GGAAGGAGAAGATGCCAG; Bax GAGCTGCAGAGGATGATTG and CCCAGTTGAAGTTGCCATC; β-actin GAACCCTAAGGCCAACCGTG and AGGCATACAGGGACAACACAGC.

2.4. Statistical analysis

Data were shown as average ± standard deviation. Data analysis was performed using the SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). Values between two or more groups were compared by independent *t*-tests. $p < 0.05$ indicated significant difference.

3. Results

3.1. Pathological examination of the organs

We collected rat heart, liver, lung, kidney specimens from the six groups. The sections were stained with HE, and inflammatory infiltration was detected under a microscope. We found significant pathological aspects in the liver and kidney of CLP group but these aspects were reduced in Esmolol group (Fig. 1). However, we did not find significant differences in inflammatory infiltration in the heart and lung sections of different groups (Fig. 2), probably due to the short duration between surgery and sacrifice.

3.2. Comparison of Bcl-2 and Bax expression in the liver and kidney

To understand the molecular mechanism underlying the pathological changes in the liver and kidney, we detected the expression of Bcl-2 and Bax. Compared to Con group, Bcl-2 mRNA expression level in the liver increased gradually in CLP group, Antibiotic group, L group, M group and H group. Bax mRNA expression in CLP group was the

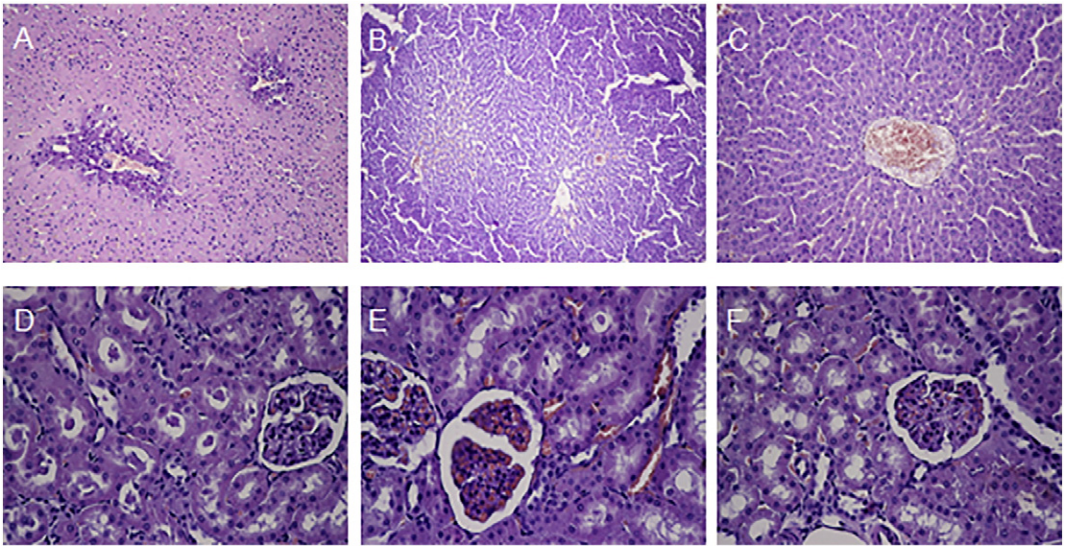

Fig. 1. Pathological analysis of the liver (A–C) and kidney (D–F). A: CLP Group, extensive hepatic ischemia, neutrophil infiltration in the hepatic sinus; B: Antibiotic Group, individual hepatic ischemia, liver sinus congestion; C: Antibiotics + Esmolol Group, normal hepatic lobule with central venous congestion. D: CLP Group, large number of tubular structures; E: Antibiotic Group, the structure of renal unit tube was less than that of sepsis group; F: Antibiotics + Esmolol Group, no nephron tubular structure. HE staining 200×.