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Infusion of esmolol attenuates lipopolysaccharide-induced myocardial dysfunction

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ABSTRACT

Background: Myocardial depression, as a well-recognized manifestation of cardiac dysfunction, often accompanies severe sepsis and septic shock. Inflammation-induced myocardial apoptosis is key to the development of sepsis-induced cardiac dysfunction. Increasing evidence suggests the anti-inflammatory role of β1-adrenergic blocker, esmolol, during lethal endotoxemia. However, the direct protective effect of esmolol on cardiomyocyte viability during sepsis is still not clear. Here, we aimed to study whether infusion of esmolol can directly inhibit myocardial apoptosis during lipopolysaccharide (LPS)-triggered inflammatory insult.

Methods: C57BL/6 mice were randomized into four groups as follows: control; esmolol infusion; LPS insult; and esmolol infusion + LPS insult. Function of left ventricle was assessed by invasive hemodynamics at 6 h after LPS insult. Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) staining, caspase-3 expression level, and the Bcl-2/Bax ratio were used to evaluate myocardial apoptosis at 6 h after LPS insult or esmolol infusion.

Results: Administration of LPS resulted in significant decrease in left ventricular end-systolic pressure, reduced maximal rate of change of left ventricular pressure, and the increase in left ventricular end-diastolic pressure. Esmolol infusion reversed LPS-induced impairment of cardiac function and reduced LPS-induced myocardial apoptosis that is associated with c-Jun N-terminal kinase (JNK) and p38 activation.

Conclusions: These data demonstrate that cardioprotection provided by esmolol infusion during LPS insult is associated with antiapoptotic effects and regulation of JNK and p38 activations.

1. Introduction

Sepsis, as a systemic response to infection, results in significant physiologic derangements, leads to multiple-organ dysfunction and death, and places a substantial financial burden on the health care system [1]. Lipopolysaccharide (LPS) as an endotoxin, one of the important inflammatory mediators in major surgical injury, is a component of the outer

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membrane of most gram-negative bacteria and plays a central role in sepsis [2]. Although much progress has been made in the treatment of systemic inflammation, septic shock remains a significant cause of postoperative morbidity and mortality. Specific antiendotoxin therapies have proved disappointing in clinical practice, especially in the intensive care unit setting [3]. LPS-induced cardiac contractile anomalies may include decreased β-adrenergic sensitivity, elevated level of inducible nitric oxide synthase, higher amount of reactive oxygen species, increased oxidative stress, and mitogen-activated protein kinase, all of which contribute to myocardial apoptosis and cardiac dysfunction [4,5]. Earlier report indicated that LPS-induced myocardial depression was significantly attenuated or ablated by elevated antioxidants including metallothionein and insulin-like growth factor I [6]. Reactive oxygen species are known to trigger mitochondrial damage and apoptosis through activation of essential stress signaling molecules including c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase [7]. Nonetheless, the exact cellular and molecular mechanisms underlying sepsis-induced cardiac depression have not been well elucidated.

β-Blocker therapy helps to control the heart rate and attenuate the deleterious effects of β-receptor stimulation by catecholamines in septic shock [8]. Moreover, recent studies demonstrated that selective β1-adrenergic blockers might possess a new therapeutic capability under hypermetabolic states such as sepsis or burn injury by attenuating catecholamine-induced metabolic alterations and excessive inflammatory responses [9]. It was also shown that infusion of the selective β1-adrenergic blocker, esmolol, during development of sepsis minimized progression of myocardial dysfunction in an *ex vivo* experimental setting [10]. The earlier report also indicated that β1-blocker therapy ameliorated septic outcome possibly through the modulation of gut mucosal integrity and local inflammatory response [9]. Although β1-blocker is suggested to have protective effect on cardiac function in sepsis, the underlying mechanism remains to be elucidated. Therefore, in the present study, we examined the protective effects of a selective β1-blocker against cardiac dysfunction and myocardial apoptosis in endotoxin (LPS)-induced sepsis in mice and assessed its regulation on activation of JNK/p38 signaling that is related to induction of myocardial apoptosis.

2. Materials and methods

2.1. Animal preparation

All experiments were conducted in accordance with Institutional Animal Care and Use Committee guidelines of Nanchang University. Male C57BL/6J mice aged 8–12 wk (Nanchang University, Jiangxi, China) were used. The animals were maintained in a pathogen-free environment with *ad libitum* access to food and water and allowed to acclimate to the environment for at least 5 d before the initiation of experimental protocols. Studies were designed to minimize discomfort and reduce the number of animals required to reach statistically significant results.

2.2. Experimental protocol

On the day of experiment, mice were injected intraperitoneally with 6 mg/kg of *Escherichia coli* O55:B5 LPS (dissolved in sterile saline) or an equal volume of pathogen-free saline (for control groups). The dosage of LPS was based on earlier reports of overt myocardial dysfunction without significant mortality [6]. Immediately after LPS insult, animals were assigned to one of two groups: an experimental group to be treated with esmolol at 6.7 mg/mL (Webster Veterinary Supply, Devens, MA) and a control group treated with saline (Baxter, Deerfield, IL). The chosen dose of esmolol allowed us to assess the overall effect of the blocker therapy while minimizing impact on hemodynamic profile. In addition, the dose of esmolol was below the threshold of inotropic effect on mice. The drug was delivered continuously via osmotic pump (ALZET, Cupertino, CA) inserted into the jugular vein fitted with small-tip mouse jugular catheters at a flow rate of 1 μL/h (6.7 μg/kg/min) for 6 h.

2.3. Hemodynamic measurements

Hemodynamic measurements were conducted after 6 h of esmolol or saline infusion. Briefly, at 6 h after infusion, animals were anesthetized with a combination of Hyponorm (Janssen Pharmaceuticals, Dublin, Ireland) and midazolam (Roche Pharmaceuticals, Dublin, Ireland). Then, a microtip pressure catheter (SPR-671, 1.4 Fr; Millar Instruments Inc, Houston, TX) was inserted into the right carotid artery under an operating microscope and advanced under pressure control into the left ventricle. After a period of stabilization, the signals were recorded continuously using a pressure conductance system coupled with a PowerLab/4SP AD converter (AD Instruments, Oxfordshire, United Kingdom). The left ventricular parameters, including heart rate, left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP), and maximal and minimal rate of change of left ventricular pressure (dP/dtmax and dP/dtmin), were analyzed by PowerLab software. Subsequently, the catheter was withdrawn into the aorta for systolic and diastolic blood pressure and heart rate measurements [11].

2.4. Caspase-3 assay

The caspase-3 activity was measured as previously described [12]. Briefly, after the hemodynamic and cardiac evaluation, 1 mL of phosphate-buffered saline was added to a flask containing myocardial homogenates, which was then centriguated at 10,000*g* at 4°C for 10 min. The supernatant was discarded, and the homogenates were lysed in 100 μL of ice-cold cell lysis buffer (50-mM Hepes, pH 7.4, 0.1% Chaps, 1-mM dithiothreitol, 0.1-mM EDTA, and 0.1% NP-40). The assay was carried out in a 96-well plate with each well containing 30 μL of cell lysate, 70 μL of assay buffer (50-mM Hepes, 0.1% Chaps, 100-mM NaCl, 10-m MDTT, and 1-mM EDTA), and 20 μL of caspase-3 colorimetric substrate Ac-DEVD-p-nitroanilide (Sigma, St. Louis, MO). The 96-well plate was incubated at 37°C for 1 h, during which time the caspase in the sample was allowed to cleave the chromophore pNA from the substrate molecule. Absorbency was detected at 405 nm, with caspase-3 activity