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Original Contribution

Butyrate pretreatment attenuates heart depression in a mice model of endotoxin-induced sepsis via anti-inflammation and anti-oxidation☆

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ABSTRACT

Objectives: The depressed heart function is the main complication to cause death of septic patients in clinic. It is urgent to find effective interventions for this intractable disease. In this study, we investigated whether butyrate could be protective for heart against sepsis and the underlying mechanism.
Methods: Mice were randomly divided into three groups. Model group challenged with LPS (30 mg/kg, i.p.) only. Butyrate group received butyrate (200 mg/kg·d) for 3 days prior to LPS administration (30 mg/kg). Normal group received saline only. 6 h and 12 h after LPS administration were chosen for detection the parameters to estimate the effects or mechanism of butyrate pretreatment on heart of sepsis.
Results: The data showed that septic heart depression was attenuated by butyrate pretreatment through improvement of heart function depression ($P < 0.01$) and reduction of morphological changes of myocardium. The overexpression of proinflammatory factors, TNF-α, IL-6 and LTB4, in heart tissues induced by sepsis was significantly alleviated by butyrate pretreatment ($P < 0.01$). As oxidative stress indicators, SOD and CAT activity, and MDA content in heart were deteriorated by LPS challenge, which was noticeably ameliorated by butyrate pretreatment ($P < 0.01$ or $P < 0.05$).
Conclusions: In conclusion, pretreatment with butyrate attenuated septic heart depression via anti-inflammation and anti-oxidation.

1. Introduction

During Gram negative bacteremia, the release of lipopolysaccharide (LPS), a component of the cell wall of bacteria, induces an exaggerated systemic response leading to multiple organ dysfunction syndrome (MODS) [1]. Sepsis is an increasing cause of morbidity and mortality, particularly in critically ill patients [2]. It is characterized by systemically increased capillary permeability and an accelerated expression of proinflammatory cytokines, such as tumor necrosis factor (TNF) [3], interleukin-6 (IL-6) [4,5] and leukotriene B4 (LTB4) [6]. It can even result in decreasing left ventricular (LV) ejection fraction, ventricular dilation, increasing cardiac output, and reducing systemic vascular resistance [7] to cause myocardial depression which is one of the most serious complications of human septic shock. However, since there are no effective specific therapies in clinic, many patients who survived the initial infection usually died relevant with myocardial injury. Therapies directing to neutralize proinflammatory cytokines or LPS by using TNF-α or LPS antibodies have been largely ineffective in clinical trials [8–10]. Therefore, it is urgent to find effective treatments to raise the survival rate of septic patients, especially with myocardial depression.

Butyrate, a 4-carbon fatty acid, is the product of bacterial fermentation in the intestine to maintain epithelial cell differentiation, inhibit cancer cells and promote differentiation of neoplastic cells [11,12]. Recent study which interested us showed that butyrate could prevent lethality of severe sepsis in rat model [13]. However, it is not clear whether butyrate can protect heart against the damages from septic shock. The objective of this study is to evaluate the cardioprotective effect of butyrate on the experimental sepsis mice model and the possible molecular mechanisms.

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2. Methods

2.1. Reagents

LPS from *Escherichia coli* (055:B5), sodium butyrate and pentobarbital sodium were purchased from Sigma-Aldrich Chemie GmbH (USA). TNF-α, IL-6 and LTB4 ELISA kits were bought from Shanghai WesTang Bio-Tech CO. LTD (Shanghai, China). Malondialdehyde (MDA) kit was got from Beyotime Institute of Biotechnology (Haimen, China). T-SOD kit and catalase (CAT) kit were purchased from Nanjing JianCheng Bioengineering Institute (Nanjing, China). Powerlab multi-functional physiological recorder (ML870) was obtained from AD Instruments Shanghai Trading Co. LTD (Shanghai, China). Light microscope (80i) was bought from Nikon Corporation (Japan). Transmission electron microscope (H-7500) was purchased from Hitachi (Japan).

2.2. Animals and groups

For all experiments, 20–25 g male ICR mice were provided by the experimental animal center of Wenzhou Medical University. Mice were free to access standard laboratory chow and water. Humidity was maintained at 50% and the temperature kept at 23 °C. Each animal was used only once in the experiment. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol including mice model experiment was approved by Laboratory Animal Ethics Committee of Wenzhou Medical University (NO. wdyw2014-0135).

Mice were randomly divided into three groups as follows: (1) normal group ($n = 20$), received an intraperitoneal injection of 0.4 ml of normal saline only. (2) model group ($n = 20$), received LPS (30 mg/kg, i.p.) only. (3) butyrate group ($n = 20$), received sodium butyrate (200 mg/kg·d, i.p.) three days before LPS administration, according to the previous study [14]. LV function was measured at 6 h and 12 h after mice challenged with LPS. Hearts were harvested for the following parameter examination.

2.3. Hemodynamic assessment of LV function

Hemodynamic parameters that we devised to measure including heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), the maximal rate of rise in LV pressure ($+dp/dt_{max}$) and the maximal rate of decline in LV pressure ($-dp/dt_{max}$). LV function was obtained at 6 h and 12 h after LPS administration. Mice were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg). Catheter was inserted into the LV via the right common carotid artery to collect data by Powerlab multi-functional physiological recorder.

2.4. Histopathological examination

Cardiac left ventricular myocardium was rapidly harvested and washed with ice-cold normal saline to be fixed with 10% buffered formalin. The fixed tissues were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin-eosin. The sections were observed under light microscope (80i).

2.5. Electron microscope examination

Cardiac left ventricular myocardium which was rapidly cut into pieces about the size 1 mm × 1 mm × 1 mm was immediately fixed in 2.5% glutaric dialdehyde for embedded in epoxy resin and sectioned at 0.1 μm. Under the transmission electron microscope (H-7500), ultrastructure changes of myocardium were observed.

2.6. Measurement of SOD and CAT activity, and MDA content in heart tissue

Heart tissues were harvested at 6 h and 12 h after LPS challenge to prepare tissue homogenate for the detection of SOD and CAT activity, and MDA content by using commercially available kits.

2.7. Determination of TNF-α, IL-6 and LTB4 level in heart tissue

Heart tissues were harvested at 6 h and 12 h after LPS administration to prepare tissue homogenate for the measurement of TNF-α, IL-6 and LTB4 by using double-antibody sandwiched ABC-ELISA with corresponding ELISA kits. Data were expressed as pg/mg protein.

2.8. Statistical analysis

Data were expressed as mean ± SD. Statistics were analyzed by Fisher's Least Significant Difference (LSD) test followed by a Dunnett's T3 multiple comparison test to determine the significant difference between each group. A value of $P < .05$ was considered to indicate statistical significance.

3. Results

3.1. Butyrate pretreatment attenuated LV function depression in septic shock induced by LPS

To study protective function of butyrate for heart during septic shock, hemodynamic parameters were obtained (Fig. 1). After 6 h of LPS challenge, HR and LVEDP were higher, while LVSP was lower by contrast with normal group ($P < 0.01$). But after 12 h of LPS administrated, HR, LVSP, $+dp/dt_{max}$ and $-dp/dt_{max}$ were all severely depressed, while LVEDP was higher than normal group ($P < 0.01$). Heart rate decreased from 541 ± 16 to 229 ± 5 times/min, LVSP fell from 98 ± 2 to 60 ± 1, $+dp/dt_{max}$ decreased from 4033 ± 575 to 1588 ± 367 mm Hg/s, $-dp/dt_{max}$ decreased from 2944 ± 286 to 798 ± 362 mm Hg/s, and LVEDP increased from 7 ± 2 to 10 ± 2 mm Hg. Satisfactorily, the frustrated LV function was obviously reversed by butyrate pretreatment. LVSP was higher in the butyrate group than model group ($P < 0.01$), while HR, $+dp/dt_{max}$ and $-dp/dt_{max}$ were lower at 6 h after LPS challenge ($P < 0.01$). These hemodynamic parameters except LVEDP in butyrate group at 12 h following LPS treatment, however, were significantly higher than those in model group ($P < .01$).

3.2. Butyrate pretreatment reduced myocardial tissue injury induced by LPS challenge

Histological evaluation of cardiac left ventricular myocardium was performed (Fig. 2A). In the normal group, the structure of cardiac muscle fibers were normal and striation was clear, no inflammatory cell infiltration was found in tissue. In the model group, however, the inter space among cardiac muscle fibers became broaden and myocardial cell edema could be observed at 6 h after LPS challenge (Fig. 2Ab). Damages of cardiac muscle fibers were more seriously at 12 h following LPS. We found more severely cardiomyocytes edema, some inflammatory cells infiltrated in heart tissue and even lysis of some cardiac muscle fibers (Fig. 2Ad). Whereas all these lesions for cardiomyocyte were alleviated by butyrate pretreatment (Fig. 2Ad/Ae). The sections showed that cardiomyocyte edema and inflammatory cell infiltration were better than model group at the same time point by contrast.

Ultrastructure of cardiomyocyte was further evaluated under an electron microscope. In the normal group, structure of intercalated disc, myofibril and mitochondria in cardiomyocyte was normal (Fig. 2B). In the model group, however, intercalated disc changed into unclear and some myofibrils were cracked, mitochondria became swelling and mitochondrial cristae damage could be observed at 6 h after LPS administration (Fig. 2Bb). We discovered that more myofibrils cracked,