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Sodium butyrate alleviates LPS-induced acute lung injury in mice via inhibiting HMGB1 release

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ABSTRACT

Sodium butyrate (SB) is a short chain 4-carbon fatty acid salt naturally exists in animal fats. Previous studies have proven that sodium butyrate has many beneficial functions such as anti-tumor and anti-inflammatory actions. In the current study we investigated the effect and possible mechanism of sodium butyrate in LPS-induced acute lung injury (ALI). ALI was induced by intratracheal administration of LPS (10 mg/kg) in male BALB/c mice. Sodium butyrate (500 mg/kg) was administered intraperitoneally 30 min prior to LPS exposure. We found that sodium butyrate significantly protected animals from LPS-induced ALI as evidenced by decreased the lung wet to dry weight ratio, total cells, neutrophils, macrophages, myeloperoxidase (MPO) activity, and lung histological damage compared to vehicle control. Sodium butyrate pretreatment markedly inhibited the production of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Furthermore, sodium butyrate pretreatment dramatically suppressed HMGB1 release and NF-κ B activation. Together, these results suggest that sodium butyrate pretreatment protects mice from LPS-induced acute lung injury, possibly through the modulation of HMGB1 and inflammatory responses.

1. Introduction

Acute lung injury (ALI) is a clinical syndrome caused by alveolar epithelial cells and capillary endothelial cell damage, resulting in diffuse lung injury known as acute respiratory distress syndrome (ARDS) [1]. The mortality rate of ALI is as high as 30%–40% and still a clinical problem to be solved, despite current advances in medical therapy [2]. Given the serious threat of ALI to human life and health, more effective drugs are needed to improve its clinical outcome [3]. Inflammatory infiltration is the main pathological manifestation of acute lung injury, accumulation of proinflammatory cells including neutrophils and macrophages and proinflammatory cytokines such as IL-6 and TNF-α cause pulmonary interstitial, alveolar edema and epithelial cell damage, finally resulting in acute hypoxic respiratory insufficiency [4]. Lipopolysaccharide (LPS), the main component of the cell wall of gram-negative bacteria, is one of the most important factors to induce pulmonary and systemic inflammatory response [5, 6]. Therefore, LPS has been widely used to establish the model of acute lung injury [7].

High-mobility group box 1 (HMGB1) is a highly conserved DNA-binding protein when secreted to the extracellular space. As a nuclear protein, HMGB1 regulates the stability of nucleosomal structures and the stability of the transcription factor binding to the target gene sequence [8, 9]. Recently, HMGB1 was identified as a proinflammatory cytokine. HMGB1 can be either actively secreted to the extracellular through the activation of macrophages and monocytes and/or passively released from necrotic cells [10, 11]. Extracellular HMGB1 activates multiple membrane receptors, including receptor for advanced glycation end products (RAGE) [12], a member of the Ig superfamily, and possibly TLR2 and TLR4, through which it contributes to the pathogenesis of inflammatory and infectious disorders such as sepsis, arthritis and ischemia reperfusion injury [13]. Recently, we also found that HMGB1 plays an important role in the development of acute hepatic injury [14]. Furthermore, extracellular HMGB1 is responsible for the progression of endotoxin-induced ALI as a late mediator and the evidence shows that HMGB1 can induce acute lung injury directly [15].

Sodium butyrate is a natural short chain 4-carbon fatty acid salt in

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animal fats. As a deacetylase inhibitor, it can increase the level of histone acetylation, thereby inhibit tumor cell proliferation, promote tumor cell senescence and apoptosis and so on [16]. Furthermore, as a potent inhibitor of HMGB1, it has been reported that sodium butyrate can suppress inflammation [17] and ameliorate organ dysfunction in a wide variety of disease models, such as sepsis, hemorrhagic shock, ischemic stroke [18] and Con A-induced hepatic injury in mice. Given the importance of HMGB1 in the initiation and progression of inflammatory processes, the aim of this study is to explore whether sodium butyrate could inhibit inflammatory cytokines especially HMGB1 release and thus improve LPS-induced ALI.

1.1. Animals

Male BALB/C mice, 6–8 weeks old and weighing 20–22 g, were provided by the Center for Animal Experiment of Wuhan University (Wuhan, China). Mice were housed for at least 1 week in the Experimental Center with a 12 h light and 12 h dark cycle prior to the experiment. All experimental procedures were carried out in accordance with Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The protocol was also approved by the Institutional Animal Care and Use Committee of Yangtze University.

1.2. Reagents

LPS (*Escherichia coli*, 055:B5) and sodium butyrate were purchased from Sigma Aldrich (Louis, USA). The myeloperoxidase (MPO) kit was obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) ELISA kits were purchased from Beijing North Institute of Biotechnology (Beijing, China). The antibodies against HMGB1 and NF-κ B were purchased from MultiSciences Biotechnology of Hangzhou (Hangzhou, China). β-actin antibody was purchased from Boster Biological Technology of Wuhan (Wuhan, China).

1.3. ALI model and experimental design

LPS was dissolved in phosphate buffer saline (PBS) at the concentration of 5μg/μl. To induce ALI, mice were given intratracheal administration of LPS at a dose of 10 mg/kg body weight. Sodium butyrate was dissolved in saline and administered into mice half an hour before via intraperitoneal injection at a dose of 500 mg/kg body weight. Mice were randomly divided into three groups: Group I (PBS group) received PBS intratracheal administration alone; Group II (LPS + sodium butyrate group), received an intraperitoneal injection of 500 mg/kg sodium butyrate followed by intratracheal LPS treatment after 30 min; Group III (LPS group) received intratracheal administration of LPS. The animals were euthanized after 24 h and used for bronchoalveolar lavage collection and tissue sampling. And mice were given intraperitoneal injection of sodium butyrate half an hour after LPS exposure as treatment group to explore the therapeutic effect of sodium butyrate. Three independent experiments were performed.

1.4. Lung wet/dry weight ratio

The right lung from each mouse was excised after euthanasia. The blood and exudate on the surface were blotted off with filter paper. The lungs were weighed to record the wet weight and then the lungs were dried in an incubator at 80 °C for 48 h to obtain the dry weight. The ratio of the wet lung to the dry lung was calculated to assess tissue edema. The ratio of the wet lung to the dry lung was got from right lung, the left lung specimens were fixed in formalin and were dehydrated for H&E staining, so both the ratio of the wet lung to the dry lung and histological examination were performed on the same sets of mice. Three independent experiments were performed.

1.5. Myeloperoxidase (MPO) activity in lung tissue

Lung tissue homogenate (1:10, w/v) was prepared in normal saline. Then the homogenate was diluted (1:1) with solution B, and the samples were assayed spectrophotometrically for MPO activity following manufacturer's instruction. The absorbance was determined at 460 nm. One unit of MPO is defined as the amount of MPO capable of degrading one micromole of peroxide per gram of wet lung tissue at 37 °C. The results are expressed as MPO units per gram of wet lung tissue. MPO was performed on independent sets of mice. Three independent experiments were performed.

1.6. Bronchoalveolar lavage fluid (BALF) collection and cells count

Bronchoalveolar lavage was collected by intratracheal injection of 1 mL PBS followed by gentle aspiration for 3 times. Recovered fluid was pooled and centrifuged at 1500 rpm for 10 min at 4 °C [1]. The BALF supernatant was preserved for the measurement of the count of IL-6 and TNF-α by ELISA. The sediment cells were resuspended in 1%BSA and total cells, neutrophils, macrophages were counted. Cell counts and ELISA for IL-6 and TNF-α were performed on the same sets of mice. Three independent experiments were performed.

1.7. Histological examination

Histopathologic evaluation was performed on mice that were not subjected to BALF collection. Mice under diethyl ether anesthesia were euthanized 24 h after LPS administration, the left lung specimens were fixed in formalin and were dehydrated, embedded in paraffin, and cut into 5 μm sections. After deparaffinization, the tissues were stained with hematoxylin and eosin, the pathological changes were observed under a light microscope. The severity of lung injury was assessed by lung pathological changes, including infiltration of inflammatory cells in air spaces or vessel walls, alveolar congestion, hemorrhage and thickness of alveolar wall/hyaline membrane formation by a blinded observer. Each factor was divided into five grades: 0 = minimal damage, 1 = mild damage, 2 = moderate damage, 3 = severe damage and 4 = maximal damage as previously described [19]. The ratio of the wet lung to the dry lung was got from right lung, the left lung specimens were fixed in formalin and were dehydrated for H&E staining, so both the ratio of the wet lung to the dry lung and histological examination were performed on the same sets of mice. Three independent experiments were performed.

1.8. Measurement of TNF-α, IL-6 in BALF

The TNF-α and IL-6 protein levels in the BALF were measured by ELISA according to the manufacturer's instructions. Cell counts and ELISA for IL-6 and TNF-α were performed on the same sets of mice. Three independent experiments were performed.

1.9. Western blot analysis

Lung tissues were homogenized and placed in a mixture of RIPA buffer and PMSF for 30 min on ice. Following BCA protein quantitative assay, the samples were loaded into a 10% SDS-PAGE gel and were electro-transferred onto a polyvinylidenerdifluoride (PVDF) membrane. After blocking for half an hour at decolorization table (80–100 rpm) with 5% nonfat dry milk at room temperature, the blots were incubated at 4°Cwith specific antibodies, including HMGB1(1:1000), β-actin (1:1000), and NF-κ B (1:500). After washes with TBST three times for 5 min, the blots were incubated with a horseradish peroxidase-conjugated secondary antibody for 1 h. After three timers washes with TBST for 10 min/wash, proteins were detected by ECL (Enhanced Chemiluminescence) plus Western blotting Detection System. Western blot was performed on independent sets of mice. Three independent