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Original Article

Berberine protects against lipopolysaccharide-induced acute respiratory distress syndrome via sirtuin-1/NF-κB pathway

Yadong Chen[1,2], Jun Luo[2], Junping Sun[1], Mingyue Zhang[1], Jianxin Wang[1]

[1]Department of Respiratory Diseases, Chinese PLA General Hospital, Beijing, China; [2]Health Division, Guard Bureau of Joint Staff Department of Central Military Commission, Beijing, China

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Abstract: Objectives: To analyze the effect of berberine on acute respiratory distress syndrome (ARDS) and clarify the underlying mechanism. Background: ARDS is a common respiratory disease. There is no standard therapeutic drug for the disease. Berberine may attenuate lung injury via inhibiting inflammation. Methods: The ARDS model was established with lipopolysaccharide administration. The lung injury was evaluated by analyzing the pulmonary pathology, edema and blood oxygenation with hematoxylin and eosin staining, wet to dry ratios and arterial blood gas analysis. The levels of inflammatory factors were determined by ELISA. To clarify the underlying mechanism, western blot was used to analyze the expression of NF-κB and sirtuin-1. Results: Berberine significantly repressed disease progression in lipopolysaccharide-induced ARDS rats, which was indicated by reduced pulmonary neutrophil infiltration, decreased wet to dry ratios and improved blood oxygenation. The effect was comparable to meprednisone treatment. The expression of TNF-α, IL-6, IL-10, ICAM-1, IL-1β and IL-8 was also inhibited by berberine treatment. Further studies showed that the sirtuin-1 mediated NF-κB pathway might be responsible, as the addition of sirtuin-1 inhibitor blocked the effect of berberine, while the agonist simulated berberine treatment. Conclusion: Berberine protects against lipopolysaccharide-induced acute respiratory distress syndrome via the sirtuin-1 pathway.

Keywords: Acute respiratory distress syndrome, berberine, sirtuin 1, NF-κB

Introduction

Acute respiratory distress syndrome (ARDS) is a common respiratory disease that is associated with 30%-60% of hospital mortality [1, 2]. The pathogenesis of the disease is complicated. Researchers have found that dysregulated inflammation might be strongly associated with the progression of ARDS [3]. However, there haven't been any standard therapeutic drugs for the treatment of this disease. In order to find the underlying mechanism, animal models were established. Among them, Lipopolysaccharide (LPS)-induced ARDS is mostly widely used [4].

Berberine (BBR) is a kind of isoquinoline alkaloid that is isolated from Berberidacea plant family [5]. It has traditionally been used as a therapeutic drug for diarrhea and gastrointestinal disorders for centuries [5]. New biological activities of BBR have been discovered in the last decades. BBR was remarkably efficient in down-regulating hyperglycemia in type 2 diabetes via the activation of AMPK pathway [6, 7]. It is also effective in neurodegenerative disease, such as Alzheimer, Parkinson and Huntington disease depending on its antioxidant activity [8, 9]. In addition, BBR has shown to be an alternative intervention for regulating lipid metabolism and the associated complications [10]. Recent studies have demonstrated that BBR could protect against lung injury induced by various agents, such as LPS, radiation or cigarette smoke, etc. [11-14]. Inhibiting the activation of NF-κB, Nrf2 signaling pathway and the secretion of inflammatory cytokines might be responsible for the effect [11, 13, 14].

As severe lung injury is the characteristic of ARDS, we postulated that BBR should also be effective in this disease. In the present study,

we verified the therapeutic potential of BBR for ARDS in a rat model, and tried to elucidate the underlying mechanism.

Materials and methods

Animals

All of the rats used in the experiments were Male Wistar weighing 200 ± 25 g (Vital River, Beijing, China). For the establishment of the ARDS model, the rats were anesthetized with pentobarbital and treated with LPS (Sigma, St Louis, MO) through intratracheal instillation (5 mg/kg) and intravenously (5 mg/kg). The rats in the control group were treated with an equal volume of vehicle. Twenty-four hours later, the ARDS rats were treated intraperitoneally for two days with meprednisone (Met, 2 mg/kg/day), BBR (30 mg/kg/day), or Met plus BBR. In order to find out the effect of SIRT1, the rats were treated intravenously with SIRT1 inhibitor (EX257, 20 mg/kg), orally with SIRT1 agonist (SRT1720, 100 mg/kg), or SIRT1 inhibitor plus BBR, one hour before LPS treatment, and once per day for next 28 d.

Arterial blood gas analysis

After blood was drawn from the carotid arteries, the arterial blood gas analysis was performed immediately with an automatic blood gas analyzer (Radiometer, Copenhagen, Denmark). The partial pressure of oxygen in arterial blood (PaO_2), the fraction of inspired oxygen (FiO_2), the partial pressure of carbon dioxide ($PaCO_2$) and oxygen saturation (O_2Sat) were analyzed. Oxygenation index was calculated as PaO_2 divided by FiO_2 (PaO_2/FiO_2).

Lung wet to dry ratios

After euthanasia, the lungs were removed from the rats and weighed immediately to obtain the wet weight. Then the lung was placed in an oven at 65°C. The dry weight was not obtained until the weight did not change. The severity of pulmonary edema was assessed with the wet to dry ratio.

Hematoxylin and eosin staining

The lung tissues were fixed with 4% paraformaldehyde, dehydrated and embedded in paraffin. Then they were cut into 5-μm-thick sections and stained with hematoxylin and eosin. The pathology of the tissue was evaluated according to the severity of inflammation, edema, hemorrhage, atelectasis, and formation of hyaline membrane.

ELISA

The lung tissue (1 mg) was homogenized thoroughly in 200 ml RIPA lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with proteinase cocktail (Roche, Basel, Swiss). The supernatant was collected after 10 min of centrifugation at 12000 g. Then, the levels of TNF-α, IL-6, ICAM-1, IL-1β and IL-8 was analyzed according to the manufactures' instructions (Shanghai Canspec Scientific Instruments Co., Ltd, Shanghai, China).

Western blot

The lung tissues were homogenized as previously indicated. The protein levels of the supernatant were tested by BCA kit (Thermo Scientific, Rockford, IL) according to the manufactures' instructions. Twenty μg of protein in each group was subjected to SDS-PAGE and was then transferred onto a PVDF membrane. The membranes were stained with antibodies against NF-κB, SIRT1, GAPDH (ProteinTech Group, Chicago, IL). Signals were detected with enhanced chemiluminescence.

Statistical analysis

Data were presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 19.0 software (IBM Corp., Armonk, NY, USA). Differences between the control and treated groups were analyzed using the Student's *t*-test. $P < 0.05$ indicated a statistically significant difference.

Results

BBR protected against LPS-induced ARDS

In the present study, Met was taken as the positive control for ARDS. As shown in **Figure 1**, LPS administration induced significant pulmonary neutrophil infiltration and hyaline membrane formation (**Figure 1A**), and increased pulmonary edema was indicated by the wet/dry ratio (**Figure 1B**). The significant changes in FiO_2, $PaCO_2$, O_2Sat and PaO_2/FiO_2 also demonstrated the establishment of ARDS in the rats (**Figure 1C**). Treatment with BBR significantly attenuated the pathological changes in LPS-induced ARDS, including reduced pulmonary

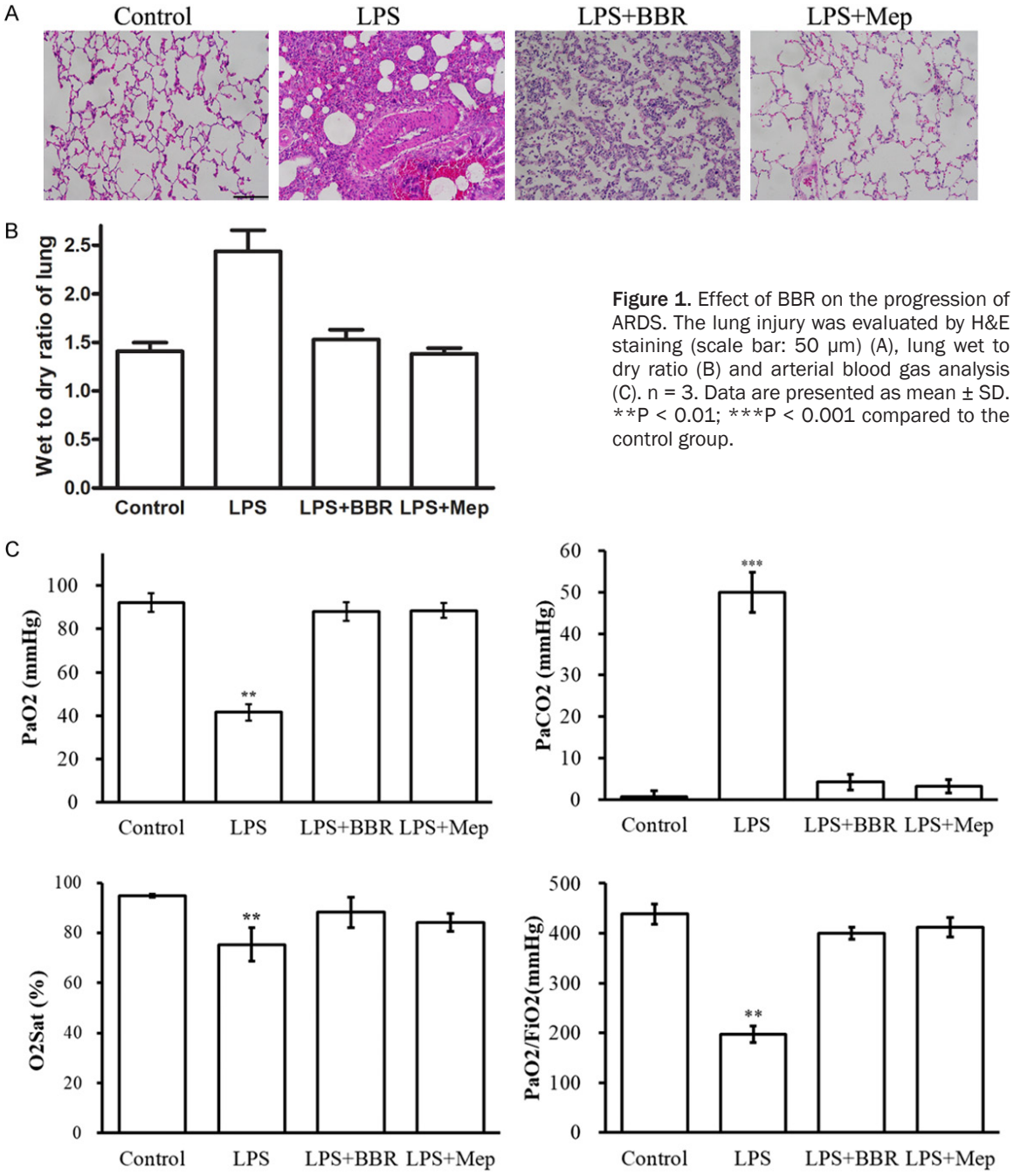

Figure 1. Effect of BBR on the progression of ARDS. The lung injury was evaluated by H&E staining (scale bar: 50 μm) (A), lung wet to dry ratio (B) and arterial blood gas analysis (C). n = 3. Data are presented as mean ± SD. **P < 0.01; ***P < 0.001 compared to the control group.

inflammation, edema, and blood oxygenation. The effect of BBR was comparable to Met, as no significant difference was found in every index.

BBR reduced the production of TNF-α, IL-6, IL-10, ICAM-1, IL-1β and IL-8 in LPS-induced ARDS

The levels of TNF-α, IL-6, IL-10, ICAM-1, IL-1β and IL-8 in the lung tissues were analyzed to evaluate the pulmonary inflammation. As shown in **Figure 2**, BBR significantly reduced the expression of TNF-α, IL-6, IL-10, ICAM-1, IL-1β and IL-8 to the control level compared to the LPS-induced ARDS group. The effect was also similar to Met treatment.

BBR inhibited the protein levels of NF-κB, SIRT1 in LPS-induced ARDS

As NF-κB was the main transcriptional regulator of inflammation, and SIRT1 was responsible for the acetylation of NF-κB, the effect of BBR on

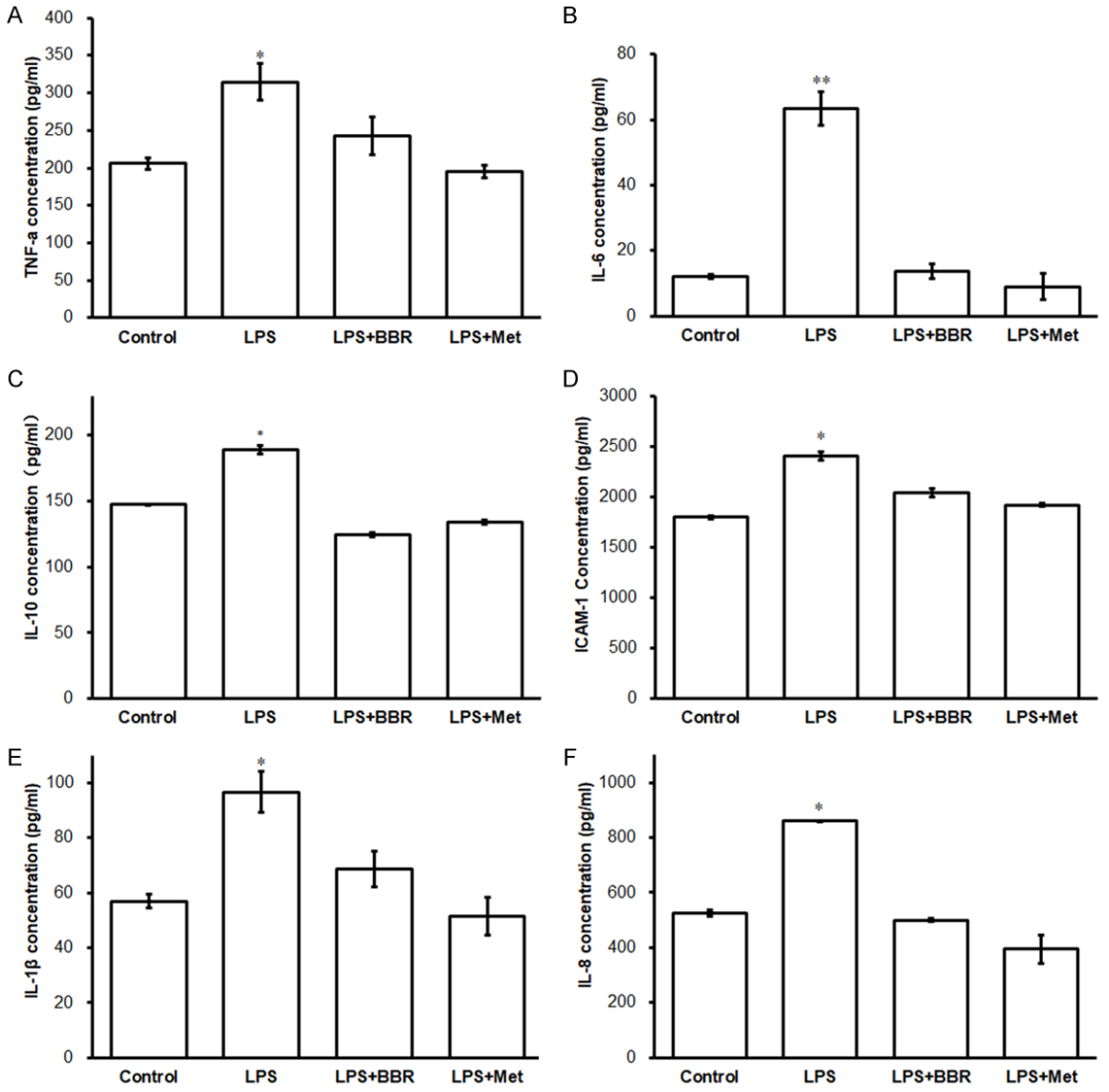

Figure 2. Effect of BBR on pulmonary inflammatory responses. The levels of TNF-α (A), IL-6 (B), IL-10 (C), ICAM-1 (D), IL-1β (E) and IL-8 (F) in the lung tissues were analyzed by ELISA. n = 3. Data are presented as mean ± SD. *P < 0.05; **P < 0.01 compared to the control group.

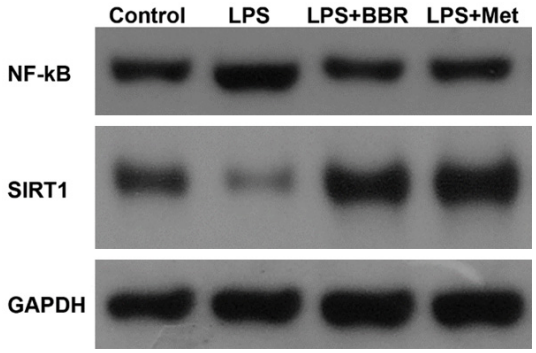

Figure 3. Effect of BBR on the expression of the SIRT1/NF-κB signaling pathway. The expression of SIRT1 and NF-κB was analyzed by western blot. GAPDH was used as a loading control.

the expression of these two factors were tested via western blot. Data showed that the relative expression of NF-κB reduced by BBR and Met compared to the LPS treated group, while the protein level of SIRT1 was upregulated in these two groups (**Figure 3**).

BBR reduced the lung injury via SIRT1 upregulation

In order to find out the role of SIRT1 in BBR treatment, the inhibitor and agonist of SIRT1 were added. As shown in **Figure 4**, the addition of SIRT1 inhibitor significantly inhibited the effect of BBR on the pathology of the lung

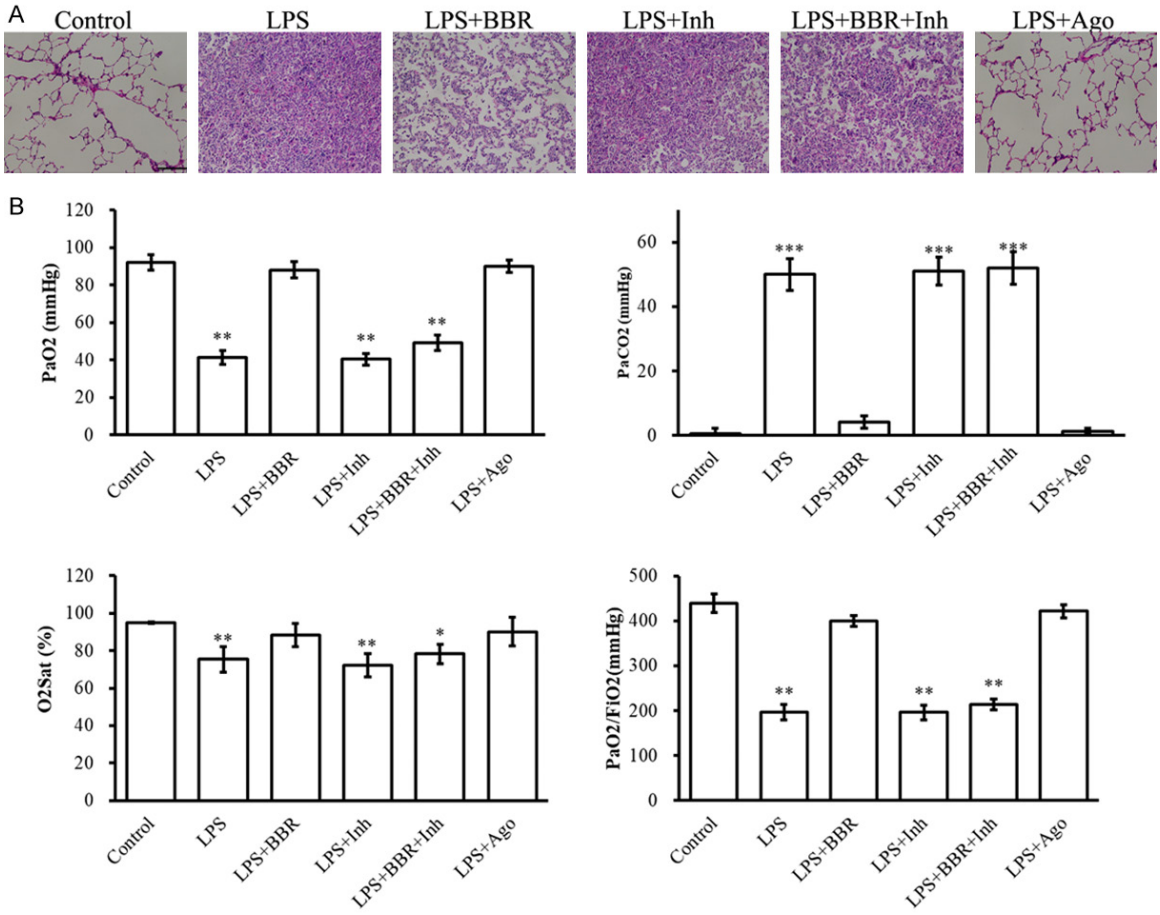

Figure 4. Effect of SIRT1/NF-κB signaling pathway on the progression of ARDS. The SIRT1 inhibitor and agonist were used to regulated the level of SIRT1. The lung injury was evaluated by H&E staining (scale bar: 50 μm) (A) and arterial blood gas analysis (B). n = 3. Data are presented as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001 compared to the control group. Inh, SIRT1 inhibitor; Ago, agonist.

(**Figure 4A**), blood oxygenation (**Figure 4B**), and the expression of inflammatory cytokines (**Figure 5**); while the treatment with SIRT1 agonist was also able to protected against LPS-induced ARDS. Furthermore, the expression of signaling pathway was also analyzed. The result was consistent to the previous data that the effect of BBR on the expression of NF-κB and SIRT1 was significantly inhibited by SIRT1 inhibitor and simulated by SIRT1 agonist (**Figure 6**).

Discussion

The present study showed that BBR was effective in attenuating the progression of ARDS. It significantly improved blood oxygenation, reduced the pulmonary edema and down-regulated the levels of inflammatory molecules in LPS-induced ARDS rats. Signaling pathway analysis showed that the activation of NF-κb was inhibited by BBR treatment and it was associated with the increase of SIRT. The addition of SIRT1 inhibitor blocked the protective effect of BBR, while SIRT1 agonist simulated the effect. These data suggested that BBR inhibited the inflammation by reducing the activation of SIRT1-NF-κB pathway, and finally attenuated the progression of ARDS.

BBR is a traditional drug that has been widely used for centuries [5]. Our data demonstrated that BBR inhibited the progression of ARDS in a rat model (**Figure 1**). The effect was consistent to the Huang et al. study in which they proved that BBR was able to attenuate ARDS progression in ARDS mice via regulating endothelial glycocalyx [15]. In the present study, we concentrated our attention on the anti-inflammatory activity of BBR instead. Accumulating evi-

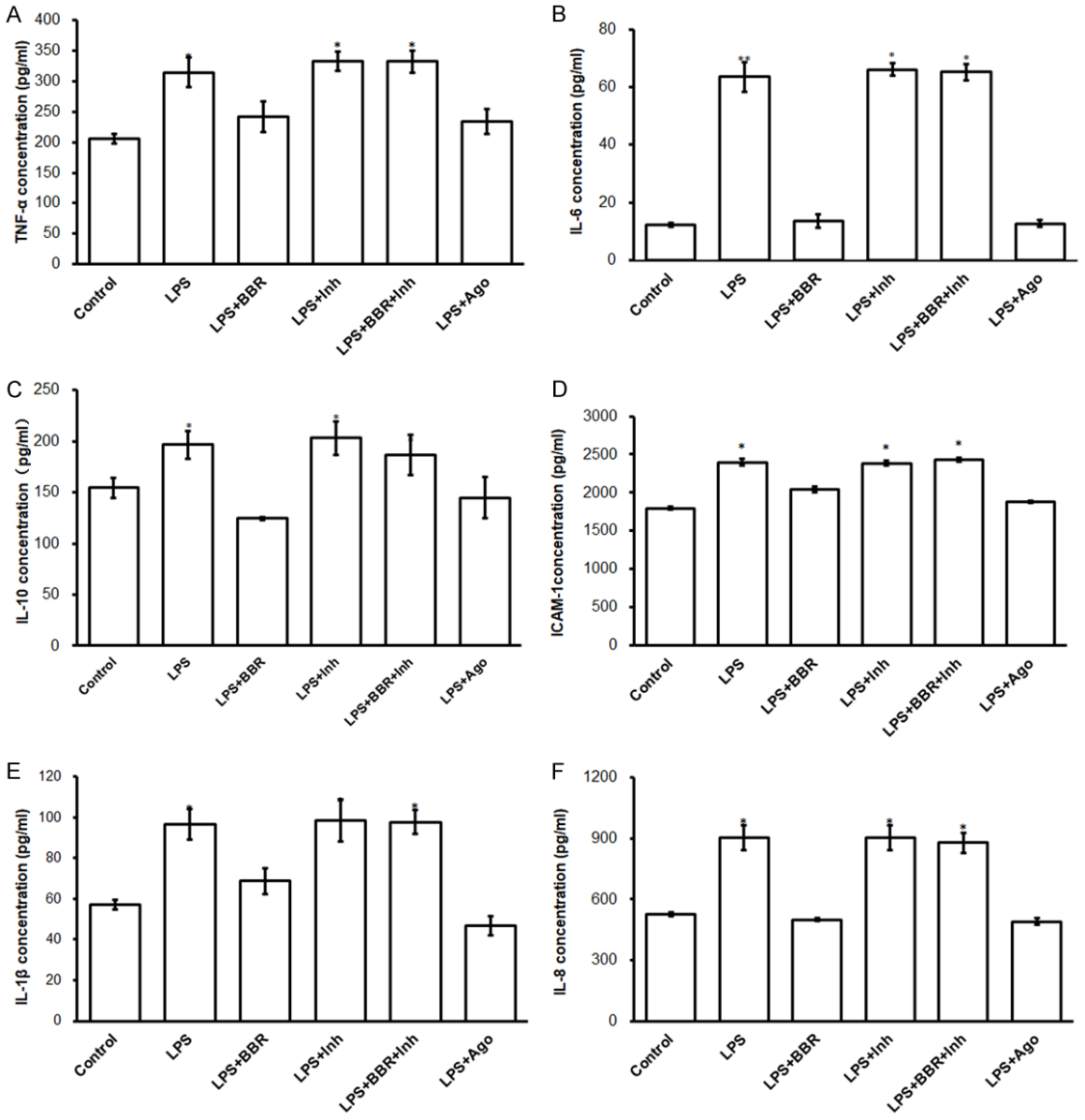

Figure 5. Effect of SIRT1/NF-κB signaling pathway on pulmonary inflammatory responses. The levels of TNF-α (A), IL-6 (B), IL-10 (C), ICAM-1 (D), IL-1β (E) and IL-8 (F) in the lung tissues were analyzed by ELISA after the treatment of BBR, SIRT1 inhibitor and agonist. Inh, SIRT1 inhibitor; Ago, agonist.

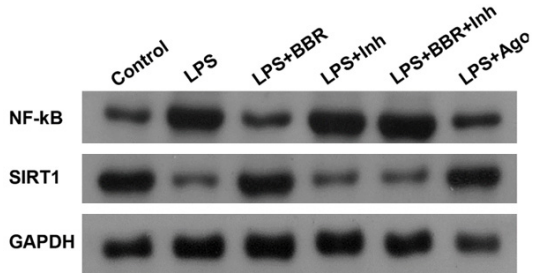

Figure 6. Effect of BBR, SIRT1 inhibitor and agonist on SIRT1/NF-κB signaling pathway. The expression of SIRT1 and NF-κB was analyzed by western blot. GAPDH was used as a loading control.

dence has demonstrated that BBR was able to reduce lung injuries by its anti-inflammatory activity [11-13, 16]. BBR treatment inhibits radiation-induced lung injury by reducing ICAM-1 and TGF-β [12]. BBR protects against cigarette smoke-induced lung injury by inhibiting neutrophil infiltration and inflammatory cytokine secretion [11]. Li et al. demonstrates that LPS-induced lung injury is reduced by inhibiting the secretion of pro-inflammatory mediators. Consistent with the above studies, we also found that BBR inhibited LPS-induced severe lung injury by inhibiting the levels of TNF-α, IL-6,

IL-10, ICAM-1, IL-1β, IL-8 (**Figure 2**). Collectively, all of the data suggests that the protective effect of BBR on lung injury might be related to different molecular mechanisms.

The NF-κB pathway plays an important role in inflammation. NF-κB remains quiescent in the cytoplasm by binding to IκB under physiological conditions. After LPS stimulation, IκB is phosphorylated and releases NF-κB to translocate to the nucleus, and leads to the expression of inflammatory factors [17]. Previous studies suggested that excessive activation of NF-κB was associated with dysregulated systemic inflammation and accelerated the progression of ARDS [3]. Agents that inhibit the pathway are always shown to be beneficial for repressing ARDS progression [18, 19]. Our data also showed that BBR significantly inhibited the expression of NF-κB and reduced lung injury (**Figure 3**). Therefore, NF-κB might be a potential therapeutic target for ARDS.

SIRT1 is a conserved NAD^+-dependent protein deacetylase. It can inhibit the acetylation of NF-κB, which results in the inhibition of inflammation [20, 21]. A series of evidence indicates that anti-inflammatory activity is related to the up-regulation of SIRT1. Previous studies have shown that BBR protected against insulin resistance in a SIRT1-dependent way [22, 23]. BBR is effective in attenuating the liver by upregulating SIRT1 expression [24]. Zhang et al. demonstrated that LPS-induced inflammation could also be reduced by BBR through the SIRT1/NF-κB pathway [25]. In the present study, we analyzed the role of SIRT1/NF-κB in ARDS by regulating SIRT1 expression. Data showed that SIRT1 inhibition significantly increased the activation of SIRT1/NF-κB pathway and aggravated lung injury in LPS-induced ARDS which was attenuated by BBR treatment (**Figures 4**-**6**). Furthermore, SIRT1 agonist could simulate the effect of BBR that alleviated the progression of ARDS. Our results were consistent with previous studies and suggested that BBR was also efficient in regulating LPS-induced ARDS through the SIRT1/NF-κB pathway.

In conclusion, our data demonstrated that BBR protected against the progression of LPS-induced ARDS via reducing the inflammation through the SIRT1/NF-κB-dependent pathway.

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Disclosure of conflict of interest

None.

Address correspondence to: Jianxin Wang, Department of Respiratory Diseases, Chinese PLA General Hospital, No. 28 Fuxing Road, Haidian District, Beijing 100853, China. Tel: +86-10-55499132; E-mail: Wangjianxin881@sohu.com

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