

ORIGINAL ARTICLE

# Piperine Ameliorates Lipopolysaccharide-Induced Acute Lung Injury *via* Modulating NF- $\kappa$ B Signaling Pathways

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**Abstract**—Piperine, one of the active components of *black pepper*, has been reported to have antioxidant and anti-inflammatory activities. However, the effects of piperine on lipopolysaccharide (LPS)-induced acute lung injury (ALI) have not been reported. Thus, the protective effects of piperine against LPS-induced ALI were investigated in this study. LPS-induced lung injury was assessed by histological study, myeloperoxidase (MPO) activity, and inflammatory cytokine production. Our results demonstrated that piperine attenuated LPS-induced MPO activity, lung edema, and inflammatory cytokines TNF- $\alpha$ , IL-6, and IL-1 $\beta$  production. Histological studies showed that piperine obviously attenuated LPS-induced lung injury. In addition, piperine significantly inhibited LPS-induced NF- $\kappa$ B activation. In conclusion, our results demonstrated that piperine had a protective effect on LPS-induced ALI. The anti-inflammatory mechanism of piperine is through inhibition of NF- $\kappa$ B activation. Piperine may be a potential therapeutic agent for ALI.

**KEY WORDS:** piperine; LPS; acute lung injury; NF- $\kappa$ B.

## INTRODUCTION

Acute lung injury (ALI) and its severe form, acute respiratory distress syndrome (ARDS), are the leading causes of mortality in critically ill patients [1]. It is characterized by severe pulmonary edema, neutrophil accumulation in the lung, and hypoxemia [2]. ALI is associated with the overproduction of inflammatory cytokines, such as TNF- $\alpha$  and IL-1 $\beta$  [3]. Mice model of lipopolysaccharide (LPS)-induced ALI has been extensively used to investigate the pathogenesis of human ALI [4, 5]. LPS induces neutrophil infiltrations and triggers inflammatory responses [6]. LPS also induces inflammatory mediator production, and these inflammatory mediators lead to severe lung injury [7]. Nowadays, the mortality of ALI remains high [8]. Thus, it is urgently needed to identify novel agents to improve the treatment of ALI/ARDS.

Piperine, one of the active components of *black pepper*, has been reported to have antioxidant and anti-inflammatory activities [9, 10]. Previous studies showed that piperine inhibited LPS-induced inflammatory mediators in RAW264.7 cells [11]. Piperine also inhibited LPS-induced maturation of bone marrow-derived dendritic cells [12]. Piperine has been reported to attenuate the severity of cerulean-induced acute pancreatitis [13]. Furthermore, piperine was found to inhibit cerebral ischemia-reperfusion-induced inflammation in middle cerebral artery occlusion rat model [14]. However, the effects of piperine on LPS-induced ALI remain unclear. In the present study, we investigated whether piperine had protective effects on LPS-induced ALI and clarified the underlying anti-inflammatory mechanism.

## MATERIALS AND METHODS

### Animals

Male BALB/c mice, weighing approximately 18 to 22 g, were obtained from the Animal Center of Jilin University (Changchun, China). All mice were housed under

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standard laboratory conditions. The mice were provided with water and food *ad libitum*. All animal experiments were performed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

### Reagents

Piperine (purity >98 %) was purchased from the National Institutes for Food and Drug Control (Beijing, China). LPS (*Escherichia coli* 0111:B4) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). ELISA kits of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were purchased from R&D Systems (Minneapolis, MN). The kit of MPO was purchased from Jiancheng Bioengineering Institute (Nanjing, China). Antibodies against p65, p-p65, I $\kappa$ B $\alpha$ , and p-I $\kappa$ B $\alpha$  were purchased from Cell Signaling Technology Inc. (Beverly, MA). Antibodies against  $\beta$ -actin and HRP-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Autogen, Bioclear, UK). All other chemicals were of reagent grade.

### Murine Model of LPS-Induced ALI

Ninety BALB/c mice were divided into six groups randomly: control group, LPS group, LPS+piperine (15, 30, and 60 mg/kg) groups. The control group and LPS group were given an equal volume of vehicle. LPS+ piperine (15, 30, and 60 mg/kg) groups were given intraperitoneally of piperine 1 h after LPS challenge. Twelve hours after LPS treatment, the mice were sacrificed and the bronchoalveolar lavage fluid (BALF) and lung tissues were collected. The choice of 12 h after LPS treatment was based on a previous study [15, 16].

### Histological Study

To assess the lung histopathological changes, the lungs were harvested, fixed in 10 % formaldehyde, embedded in paraffin, and sliced into 5- $\mu$ m sections. Then, the sections were stained with hematoxylin and eosin (H&E). The pathological changes of lung tissues were observed under a light microscope.

### Measurement of Lung W/D Ratio

The lung wet/dry (W/D) weight ratio was used to assess the edema of the lung. Briefly, the right lungs were harvested and weighted to obtain the 'wet' weight. To obtain the 'dry' weight, the lungs were kept in an oven at 60 °C for 48 h. The lung W/D ratio was calculated by dividing the wet weight by the dry weight.

### MPO Assay

The activity of lung MPO was measured by MPO kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Briefly, the lung tissue was collected 12 h after LPS treatment and 100 mg lung tissue was homogenized. Subsequently, 0.9 ml homogenate and 0.1 ml of reaction buffer was heated at 37 °C and determined in absorbance at 460 nm.

### ELISA Assay

The levels of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in the BALF were examined by commercially available ELISA kits (R&D, Minneapolis, MN, USA) according to the manufacturer's instructions.

### Western Blot Analysis

Twelve hours after LPS treatment, the lungs were collected, homogenized, and incubated with lysis buffer which includes a protease inhibitor cocktail (Sigma). After protein concentration was determined through BCA method, equal amounts (50  $\mu$ g) of proteins were separated by 10 % SDS-PAGE. Then, the proteins were transferred onto a PVDF membrane. After blocking with 5 % nonfat dry milk, the membrane was probed with primary antibody at 4 °C overnight. After washing, the membrane was probed with horseradish peroxidase-conjugated second antibody for 2 h. The detection of labeling proteins were detected with enhanced-chemiluminescence Western blot detection kits.

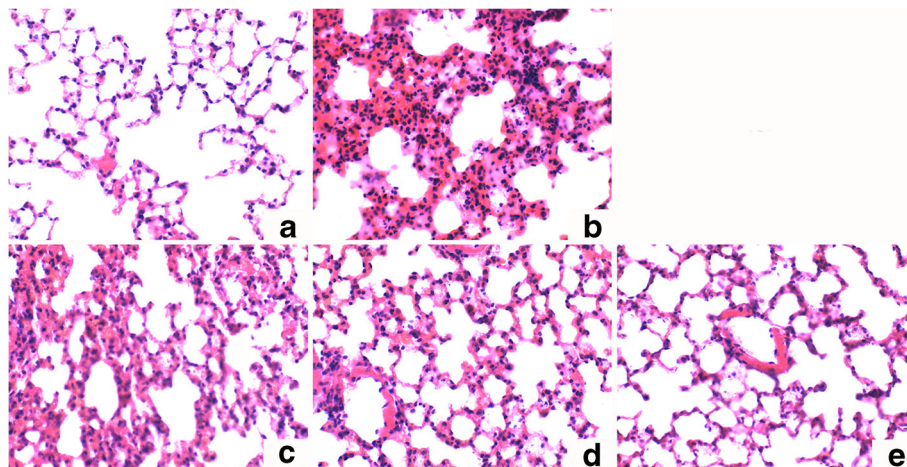
### Statistical Analysis

The values were expressed as the mean $\pm$ SD of three independent experiments. Statistically significant differences for multiple groups were assessed by ANOVA, followed by Dunnett's test. Statistical significance was accepted  $p < 0.05$  or  $p < 0.01$ .

## RESULTS

### Effects of Piperine on LPS-Mediated Lung Histopathologic Changes

Histopathological analysis was used to investigate the protective effects of piperine on LPS-induced ALI. As shown in Fig. 1, the lung section of the control group

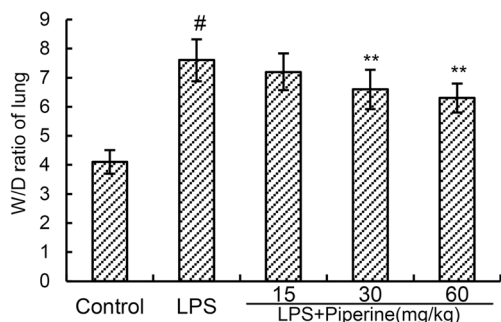


**Fig. 1.** Effects of piperine on histopathological changes in lung tissues in LPS-induced ALI mice. Mice were given an intraperitoneal injection of piperine (15, 30, and 60 mg/kg) 1 h after administration of LPS. Representative histological changes of lung obtained from mice of different groups. **a** Control group, **b** LPS group, **c** LPS+piperine (15 mg/kg) group, **d** LPS+piperine (30 mg/kg) group, **e** LPS+piperine (60 mg/kg) group (hematoxylin and eosin staining, magnification  $\times 200$ ).

displayed normal structure. The lung section of LPS-treated group showed obvious histopathological changes, including interstitial edema, hemorrhage, thickening of the alveolar wall, and inflammatory cells infiltration (Fig. 1b). However, treatment of piperine attenuated the severity of lung injuries induced by LPS (Fig. 1c–e).

**Piperine Inhibits LPS-Induced Lung W/D Ratio**

The severity of lung edema was measured by lung W/D ratio. As shown in Fig. 2, there was a significant increase in the lung W/D ratio of LPS-treated group when compared with the control group. However, compared with LPS group, the levels of W/D ratio decreased in piperine-treated groups.



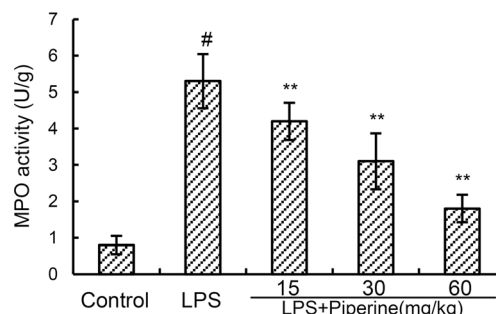
**Fig. 2.** Effects of piperine on the lung W/D ratio of LPS-induced ALI mice. The lung wet/dry weight ratio was determined at 12 h after LPS challenge. The values presented are the means  $\pm$  SEM ( $n=12$  in each group) of three independent experiments. *Octothorpe*,  $p < 0.01$  vs. control group,  $**p < 0.01$  vs. LPS group.

**Effects of Piperine on MPO Activity**

MPO activity was used to assess neutrophil accumulation in lung tissues. As shown in Fig. 3, MPO activity significantly increased in the LPS-treated group than in the control group. However, LPS-induced MPO activity was dose-dependently inhibited by treatment of piperine.

**Effects of Piperine on TNF- $\alpha$ , IL-6, and IL-1 $\beta$  Production**

Twelve hours after LPS treatment, the BALF were collected and the levels of inflammatory cytokines TNF- $\alpha$ , IL-6, and IL-1 $\beta$  were detected by ELISA. The results

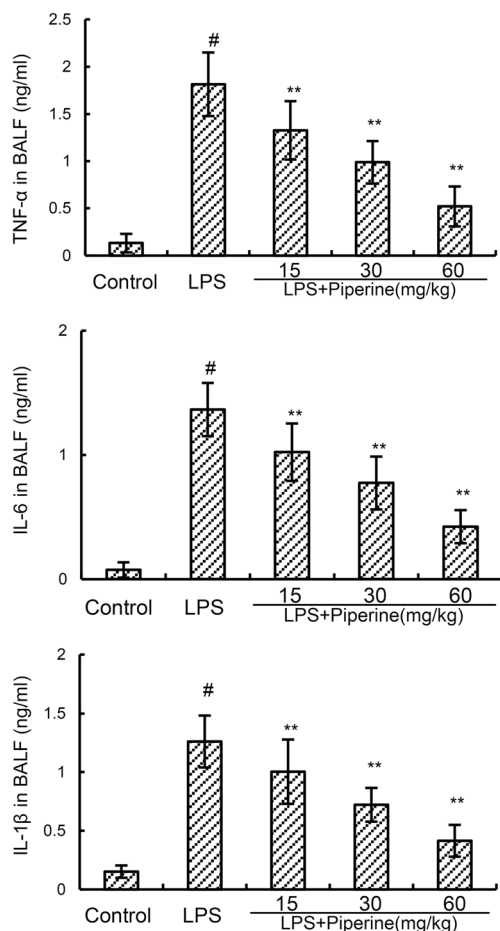


**Fig. 3.** Effects of piperine on MPO activity in lung tissues of LPS-induced ALI. MPO activity was determined at 12 h after LPS administration. The values presented are the mean  $\pm$  SEM ( $n=12$  in each group) of three independent experiments. *Octothorpe*,  $p < 0.01$  vs. control group;  $**p < 0.01$  vs. LPS group.

showed that the levels of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  increased significantly after LPS treatment. However, treatment of piperine significantly inhibited LPS-induced inflammatory cytokines TNF- $\alpha$ , IL-6, and IL-1 $\beta$  production in a dose-dependent manner (Fig. 4).

#### Effects of Piperine on LPS-Induced NF- $\kappa$ B Activation

The effects of piperine on LPS-induced NF- $\kappa$ B activation were detected by Western blotting in this study. As shown in Fig. 5, LPS significantly induced NF- $\kappa$ B activation and I $\kappa$ B $\alpha$  degradation. However, piperine dose-dependently inhibited LPS-induced NF- $\kappa$ B activation and I $\kappa$ B $\alpha$  degradation.



**Fig. 4.** Effects of piperine on TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 production in the BALF of LPS-induced ALI mice. BALF was collected at 12 h following LPS challenge to analyze the inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. The values presented are mean  $\pm$  SEM ( $n=12$  in each group) of three independent experiments. *Ocotohorpe*,  $p<0.01$  vs. control group;  $**p<0.01$  vs. LPS group.

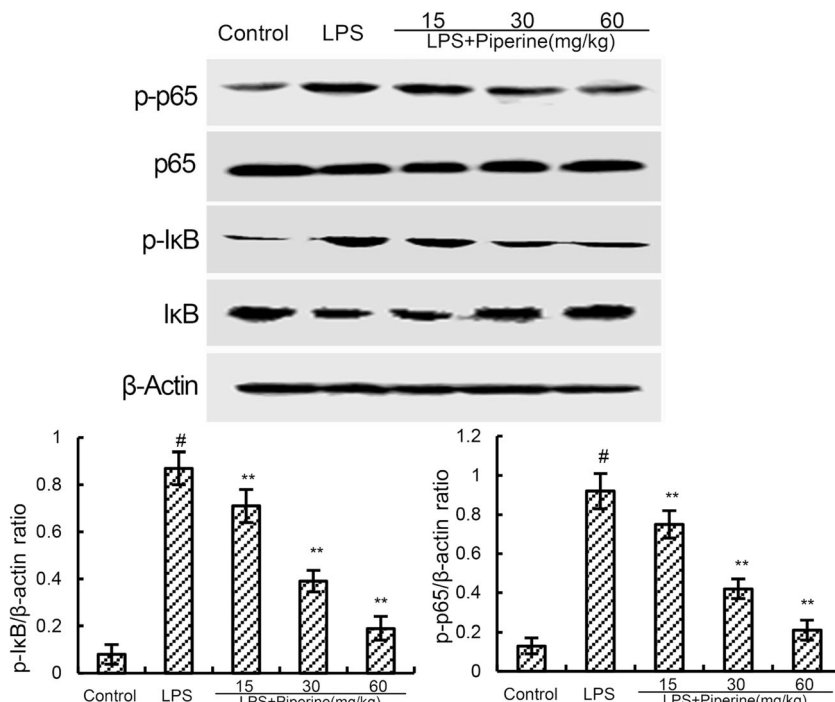
#### DISCUSSION

Piperine, one of the active components of *black pepper*, has been reported to have anti-inflammatory activities. In the current study, we found that piperine had a protective effect on LPS-induced ALI. Treatment of piperine attenuated LPS-induced lung edema, infiltration of neutrophils in the lung, and inflammatory cytokine production. These results suggested that piperine might be a potential therapeutic agent for ALI.

Treatment of LPS caused severe lung injury in mice as evidenced by interstitial edema and infiltration of neutrophils [17, 18]. In this study, we investigated the effects of lung edema by measuring lung W/D ratio. Our results showed that piperine dose-dependently inhibited LPS-induced lung W/D ratio. MPO activity, a marker of neutrophil influx into tissue, was used to assess infiltration of neutrophils in this study [19]. Our results showed that treatment of piperine significantly inhibited LPS-induced MPO activity. Histopathological analysis showed that piperine significantly inhibited LPS-induced infiltration of neutrophils in the lung. Histopathological analysis also showed that piperine remarkably attenuated LPS-induced lung pathological changes. Taken together, these results of this study suggested that piperine exhibited protective effects against LPS-induced ALI in mice.

Inflammatory cytokines played an important role in the development of lung injury [3]. Among these cytokines, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were known to be important inflammatory mediators that caused ALI [20, 21]. Meanwhile, previous studies showed that the production of inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 increased in patients with ALI [22]. These inflammatory cytokines initiated and implied the inflammatory responses and led to lung injury [23]. In this study, we found that piperine significantly suppressed LPS-induced inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 production. NF- $\kappa$ B is a transcription factor that exists in an inactive form in the cytoplasm by its inhibitory proteins I $\kappa$ B [24, 25]. Once stimulation, NF- $\kappa$ B is released from the I $\kappa$ B and translocate from the cytoplasm to the nucleus to regulate TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 gene transcription [26, 27]. To investigate the anti-inflammatory mechanism of piperine, the effects of piperine on LPS-induced NF- $\kappa$ B activation were detected. The results showed that piperine dose-dependently inhibited LPS-induced NF- $\kappa$ B activation.

In conclusion, piperine exerted its protective effects on LPS-induced ALI by inhibiting lung edema, neutrophil



**Fig. 5.** Piperine inhibits LPS-induced NF- $\kappa$ B activation and I $\kappa$ B $\alpha$  degradation. The values presented are the means  $\pm$  SEM of three independent experiments. *Octothorpe*,  $p < 0.05$  vs. control group;  $**p < 0.01$  vs. LPS group.

infiltration, and inflammatory cytokines production. These data suggested that piperine might be a therapeutic drug in the prevention and treatment of ALI. The protective effects of piperine on LPS-induced ALI were investigated in mice. Whether piperine has protective effects on humans remains unclear. However, further and more comprehensive studies are needed before piperine is used for the clinical practice.

**Conflict of Interest.** The authors declare that they have no competing interests.

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