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Paeonol attenuates acute lung injury by inhibiting HMGB1 in lipopolysaccharide-induced shock rats

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ABSTRACT

High-mobility group box 1 (HMGB1) is a highly conserved DNA-binding nuclear protein that facilitates gene transcription and the DNA repair response. However, HMGB1 may be released by necrotic cells as well as activated monocytes and macrophages following stimulation with lipopolysaccharide (LPS), interleukin-1β (IL-1β), or tumor necrosis factor-α (TNF-α). Extracellular HMGB1 plays a critical role in the pathogenesis of acute lung injury (ALI) through activating the nuclear transcription factor κB (NF-κB) P65 pathway, thus, it may be a promising therapeutic target in shock-induced ALI. Paeonol (Pae) is the main active component of *Paeonia suffruticosa*, which has been used to inhibit the inflammatory response in traditional Chinese medicine. We have proven that Pae inhibits the expression, relocation and secretion of HMGB1 in vitro. However, the role of Pae in the HMGB1-NF-κB pathway remains unknown. We herein investigated the role of Pae in LPS-induced ALI rats. In this study, LPS induced a marked decrease in the mean arterial pressure (MAP) and survival rate (only 25% after 72 h), and induced severe pathological changes in the lung tissue of rats, which was accompanied by elevated expression of HMGB1 and its downstream protein NF-κB P65. Treatment with Pae significantly improved the survival rate (> 60%) and MAP, and attenuated the pathological damage to the lung tissue in ALI rats. Western blotting revealed that Pae also inhibited the total expression of HMGB1, NF-κB P65 and TNF-α in the lung tissue of ALI rats. Moreover, Pae increased the expression of HMGB1 in the nucleus, inhibited the production of HMGB1 in the cytoplasm, and decreased the expression of P65 both in the nucleus and cytoplasm of lung tissue cells in LPS-induced ALI rats. The results were in agreement with those observed in the in vitro experiment. These findings indicate that Pae may be a potential treatment for ALI through its repression of the HMGB1-NF-κB P65 signaling pathway.

1. Introduction

Endotoxemia, which is mainly caused by lipopolysaccharide (LPS) and other bacterial products, usually causes acute lung injury (ALI), and may even lead to multiple organ failure [1]. A previous epidemiological investigation revealed that ~50% of endotoxin shock cases developed ALI, which is considered as a severe respiratory disorder, characterized pathologically by increased capillary permeability and excessive lung tissue inflammation. ALI may further progress into its severe form, acute respiratory distress syndrome (ARDS), which is one of the leading causes of mortality in endotoxin shock [2]. The treatment options for endotoxin shock with ALI remain limited, and there is an urgent need to develop novel therapies to control this condition [3].

High-mobility group box 1 (HMGB1) was initially identified as a conserved, abundant and ubiquitous chromatin-associated protein, which is indispensable for the survival of animals, as HMGB1 knockout mice cannot survival after birth [4]. However, HMGB1 is a damage-associated molecular pattern (DAMP) secreted by monocytes and macrophages following stimulation by inflammatory factors [4]. Extracellular HMGB1 was confirmed as a critical late pro-inflammatory

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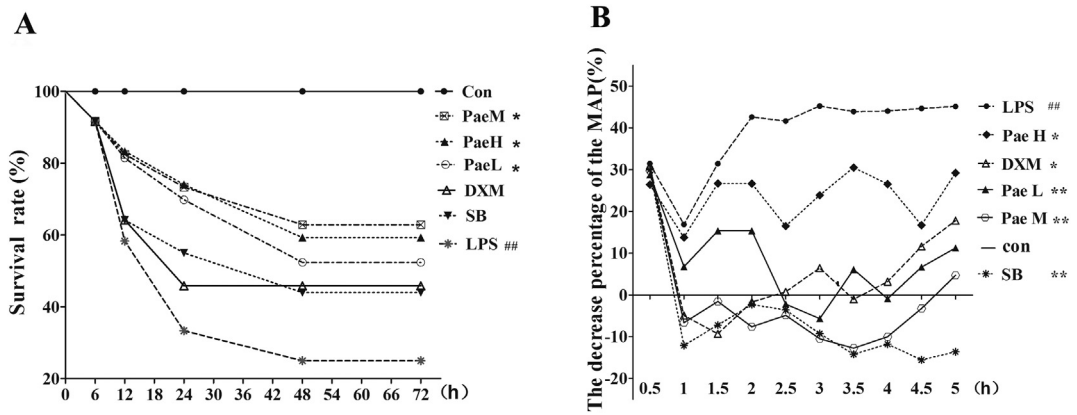

Fig. 1. Effect of Pae on survival and MAP in rats with LPS-induced shock ALI (n = 12).
(A) The Kaplan-Meier method was used to compare the survival rates among different groups at the 6-, 12-, 24-, 48- and 72-h time points. (B) The MAP of LPS-induced shock rats was recorded every 30 min for 5 h. $^{\#\#}P < 0.01$ vs. control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. LPS group. Pae, paeonol; MAP, mean arterial pressure; LPS, lipopolysaccharide; ALI, acute lung injury.

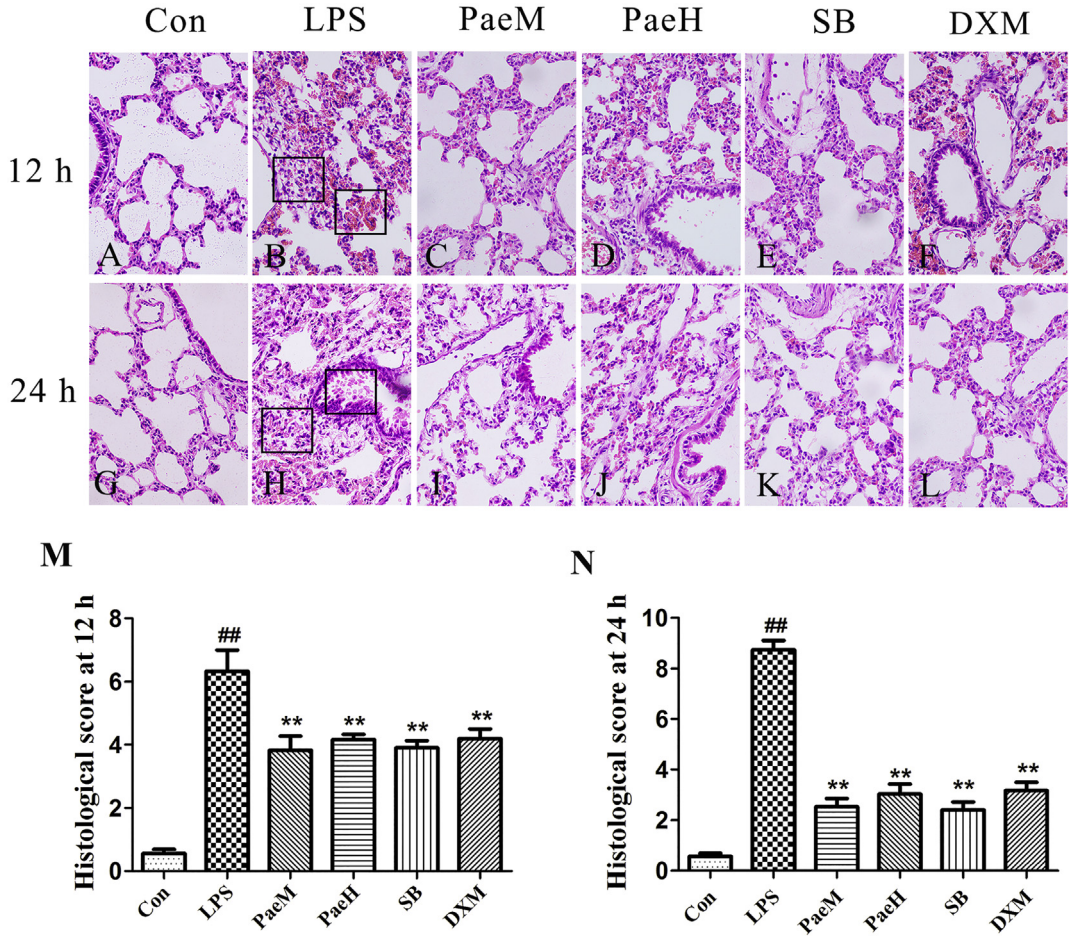

Fig. 2. Effect of Pae on lung histological alterations in rats with LPS-induced shock ALI (HE staining; magnification, ×400).
(A) HE staining of normal lung tissues revealed integral alveolar structure and clear alveolar spaces at the 12-h time point. (B) Thickened alveolar septa, pulmonary capillary hyperemia and notable inflammatory cell infiltration were readily identified after a 12-h stimulation by LPS. Pae (C and D), SB (E) and DXM (F) alleviated hyperemia and inflammatory cell infiltration. Compared with the control group (G), bronchiolar hemorrhage and pulmonary alveolar consolidation were observed after LPS induction for 24 h (H). Pae (I and J) substantially attenuated alveolar consolidation and structural damage, as did SB (K) and DXM (L). The histological changes were scored at the 12-h (M) and 24-h (N) time points (n = 6). $^{\#\#}P < 0.01$ vs. control group; $^{**}P < 0.01$ vs. LPS group. Pae, paeonol; PaeM, medium-dose Pae group; PaeH, high-dose Pae group; Con, control; LPS, lipopolysaccharide; ALI, acute lung injury; HE, hematoxylin and eosin; SB, sodium butyrate; DXM, dexamethasone.