

Pachymic acid improves survival and attenuates acute lung injury in septic rats induced by cecal ligation and puncture

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Abstract. – **OBJECTIVE:** The purpose of this study was to elucidate the possible beneficial effects of pachymic acid (PA) on acute lung injury (ALI) in a rat model of sepsis.

MATERIALS AND METHODS: A rat model of sepsis induced by cecal ligation and puncture (CLP) was used. Rats were randomly divided into five groups: sham, CLP, CLP + PA 1 mg/kg, CLP + PA 5 mg/kg, CLP + PA 10 mg/kg. CLP + PA groups received PA by intraperitoneal injection daily for consecutive 3 days, respectively, and the rats in sham and CLP groups were given equivalent volume of olive oil. We killed the animals 12 h after CLP and collected blood samples to determine PaO₂, PaCO₂, tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and IL-6. Lung samples were taken for wet/dry weight ratios and histologic assessment. Meanwhile, the levels of lung tissue myeloperoxidase (MPO), malondialdehyde (MDA) and superoxide dismutase (SOD) were determined.

RESULTS: The results revealed that PA treatment significantly improved the survival of septic rats and attenuated CLP-induced ALI. In PA-treated rats, the wet/dry weight ratios and the serum levels of TNF- α , IL-1 β and IL-6 were down-regulated compared with the CLP group. PA also markedly decreased MDA and MPO contents and increased SOD level.

CONCLUSIONS: These findings indicate that PA administration ameliorates ALI in a rat model of sepsis induced by CLP.

Key Words:

Pachymic acid, Sepsis, Acute lung injury, Inflammation, Oxidative stress.

functions and even death¹⁻³. Despite extensive therapeutic approaches, sepsis remains a leading cause of high mortality affecting millions of individuals worldwide, ranging from 20.7% to 45.7%⁴. Researchers have demonstrated that acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) is highly related to sepsis^{5,6}. Hence, it is urgent to search for novel and effective therapies for sepsis and septic complications. Pathophysiology of ALI/ARDS is characterized by complex mechanisms, including cells of inflammation, lung tissue cells, cytokines, and chemokines⁷. The lung is the first failure organ induced by sepsis, and the inflammatory response plays a central role in the pathogenesis of injury and in the repair process in ALI/ARDS⁸. More experimental data suggested that an anti-inflammatory compound has a potential preventive and therapeutic effect on sepsis and septic complications^{9,10}. Natural products are one of the main sources for discovery of new drug compounds. Pachymic acid (PA) is a lanostane-type triterpenoid from *P. cocos*, which is also called Fuling in Chinese medicine¹¹. PA possesses various pharmacological potentials, including anti-inflammatory, anti-apoptotic, and anti-cancer effects¹²⁻¹⁴. Thus, in the present study, we assessed whether treatment with the PA can reduce ALI in rats with cecal ligation and puncture (CLP)-induced polymicrobial sepsis.

Materials and Methods

Materials

PA was purchased from National Institute for the Control of Pharmaceutical and Biological Products (purity > 98%, HPLC, Beijing, China).

Introduction

Sepsis is a complex syndrome characterized by dysregulated systemic inflammatory response, which can result in multiple organ dys-

Animals

Male Wistar rats (weighing 280 g to 300 g; Experimental Animal Center of Suzhou Aiermaite technology Co. Ltd., SPF grade, Certificate No. SCXK20140007) were housed under specific pathogen-free conditions with a 12 h light/dark cycle at 22°C to 24°C and free access to water and food. All animal experiments were approved by the Committee on the Ethics of Animal Experiments of Yantaishan Hospital.

Experiment Protocol

Rats were randomly divided into five groups: sham, CLP, CLP + PA 1 mg/kg, CLP + PA 5 mg/kg, CLP + PA 10 mg/kg. Each group was assigned to 20 rats. Before CLP surgery, CLP + PA group received PA by intraperitoneal injection daily for consecutive 3 days, respectively, and the rats in sham and CLP groups were given equivalent volume of olive oil.

Sepsis Model Establishment

After an overnight fast, rats were anesthetized with an i.p. mixture of 100 mg/kg ketamine and 5 mg/kg xylazine. After sterilization, a 2-cm ventral midline abdominal incision was made and the cecum was then gently isolated and the ligated part of the cecum was then punctured twice with a 16-gauge needle. Finally, the incision was closed, and 3-ml/100 g body weight of pre-warmed sterile saline was administered s.c. No antibiotics were administered. Survival was monitored for 72 h.

Determination of the Lung Wet/dry Weight Ratio

The lung tissues were cleaned of bloodstains with absorbent paper, weighed wet, torrefied in a 75°C thermostatic baking oven for 72 h. The ratio of the wet lung to the dry lung was calculated to assess tissue edema.

Arterial Blood Gas

We drew arterial blood (0.5 ml) and measured arterial blood gas (ABG) levels (PaO_2 and PaCO_2) with an analyzer (Bayer Healthcare LLC, East Walpole, MA, USA).

Serum Cytokines Analysis

The whole blood was centrifuged at 1500 rpm for 10 min at 4°C and the serum was collected. The levels of tumor necrosis factor (TNF)- α , interleukin-1 β (IL-1 β) and IL-6 in the serum were measured by Enzyme-Linked Immunosorbent

Assay (ELISA) kits (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China), according to the manufacturer's instructions.

Biochemical Determination in Lung Tissues

Lung proteins were extracted with protein extraction kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. The contents of myeloperoxidase (MPO), malondialdehyde (MDA) and the activity superoxide dismutase (SOD) were measured according to the instructions of detection kits (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Histopathological Evaluation

To evaluate the histologic alterations, the lungs were fixed, embedded in paraffin wax, sectioned into 5- μm pieces, and stained with hematoxylin and eosine (H&E). The pathologic changes in the lung tissues were observed under a light microscope (DP73; Olympus, Tokyo, Japan).

Statistical Analysis

All data were expressed as mean $\pm$ standard deviation (SD). SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analysis. Statistical comparison of multiple groups was performed by one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. $p < 0.05$ was considered to be statistically significant.

Results

Effect of PA on Survival Rate

To examine the effect of PA on survival rates, rats were monitored every 12 h for 72 h after CLP. The survival rate was recorded (Figure 1). All rats in the CLP group were executed at 72 h after undergoing CLP surgery. In contrast, PA 1 mg/kg treatment significantly increased the survival rate by 50%, PA 5 mg/kg treatment significantly increased the survival rate by 70%, and PA 10 mg/kg treatment significantly increased the survival rate by 80% at 72 h compared with the CLP group ($p < 0.01$). No death of rats was observed in the sham group after CLP.

Lung Wet/dry Weight Ratio

Compared with the sham group, wet/dry weight ratio increased significantly in the CLP group (from 4.92 ± 0.12 to 7.68 ± 0.21 ,

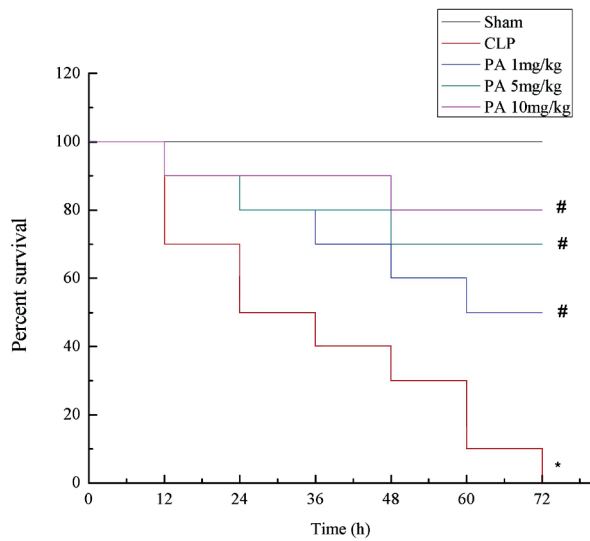


Figure 1. Effect of PA on CLP-induced mortality. PA treatment markedly improved the survival of septic rats. * $p < 0.01$ vs. sham group, # $p < 0.01$ vs. CLP group ($n = 10$).

$p < 0.05$). PA treatment was found to significantly reduce lung wet/dry weight ratio in the CLP-induced ALI in rats (Figure 2) ($p < 0.05$ in each group).

Effect of PA on ABG

As shown in Figure 3, CLP-induced significant alterations in arterial blood gas (ABG), among which PaO_2 was significantly lower ($p < 0.05$), whereas PaCO_2 was significantly higher ($p < 0.05$) after CLP operations. However, administration of PA reversed the reduction in

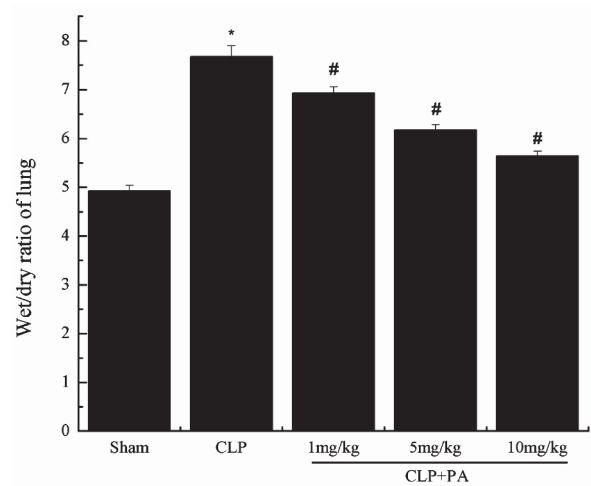


Figure 2. PA ameliorates CLP-induced W/D ratio changes in the lung. Data were shown as mean $\pm$ SD. * $p < 0.05$ vs. sham group, # $p < 0.05$ vs. CLP group.

PaO_2 compared with the CLP group ($p < 0.05$ in each group) and reversed the increase in PaCO_2 induced by CLP compared with the CLP group ($p < 0.05$ in each group).

Effect of PA on Inflammation Induced by CLP in Serum

To explore the effect of PA on the inflammatory responses in the CLP-induced ALI rats, we measured several serum cytokine levels. As shown in Figure 4, the levels of $\text{TNF-}\alpha$, $\text{IL-1}\beta$ and IL-6 in CLP group were significantly higher than that in the sham group (from 30.92 ± 2.93 to 72.37 ± 5.29 for $\text{TNF-}\alpha$, from 31.26 ± 1.87 to 56.25 ± 3.57 for $\text{IL-1}\beta$, and from 389.18 ± 49.38 to

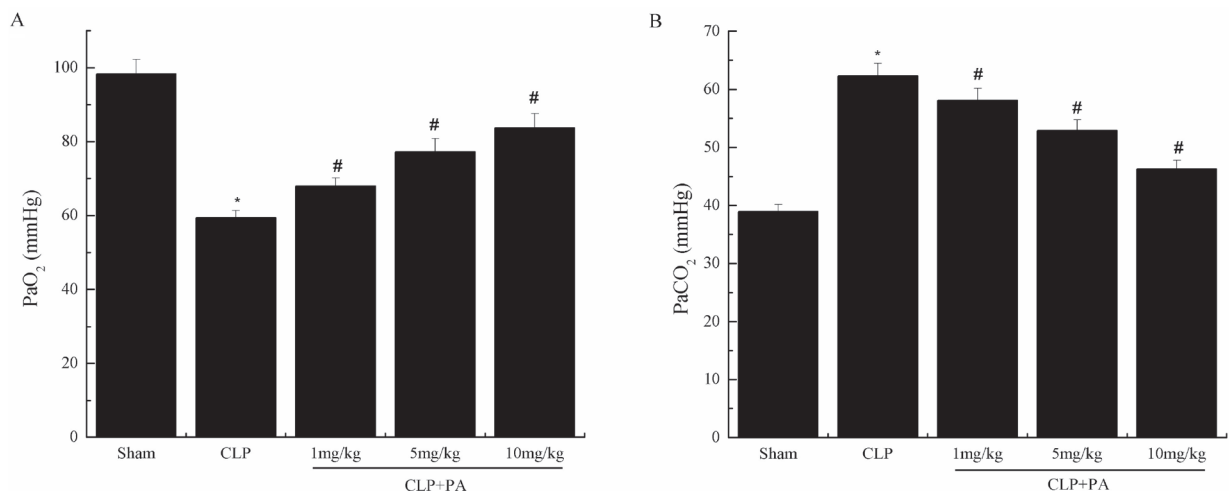


Figure 3. Effect of PA on ABG (PaO_2 and PaCO_2). Data were shown as mean $\pm$ SD. * $p < 0.05$ vs. sham group, # $p < 0.05$ vs. CLP group.

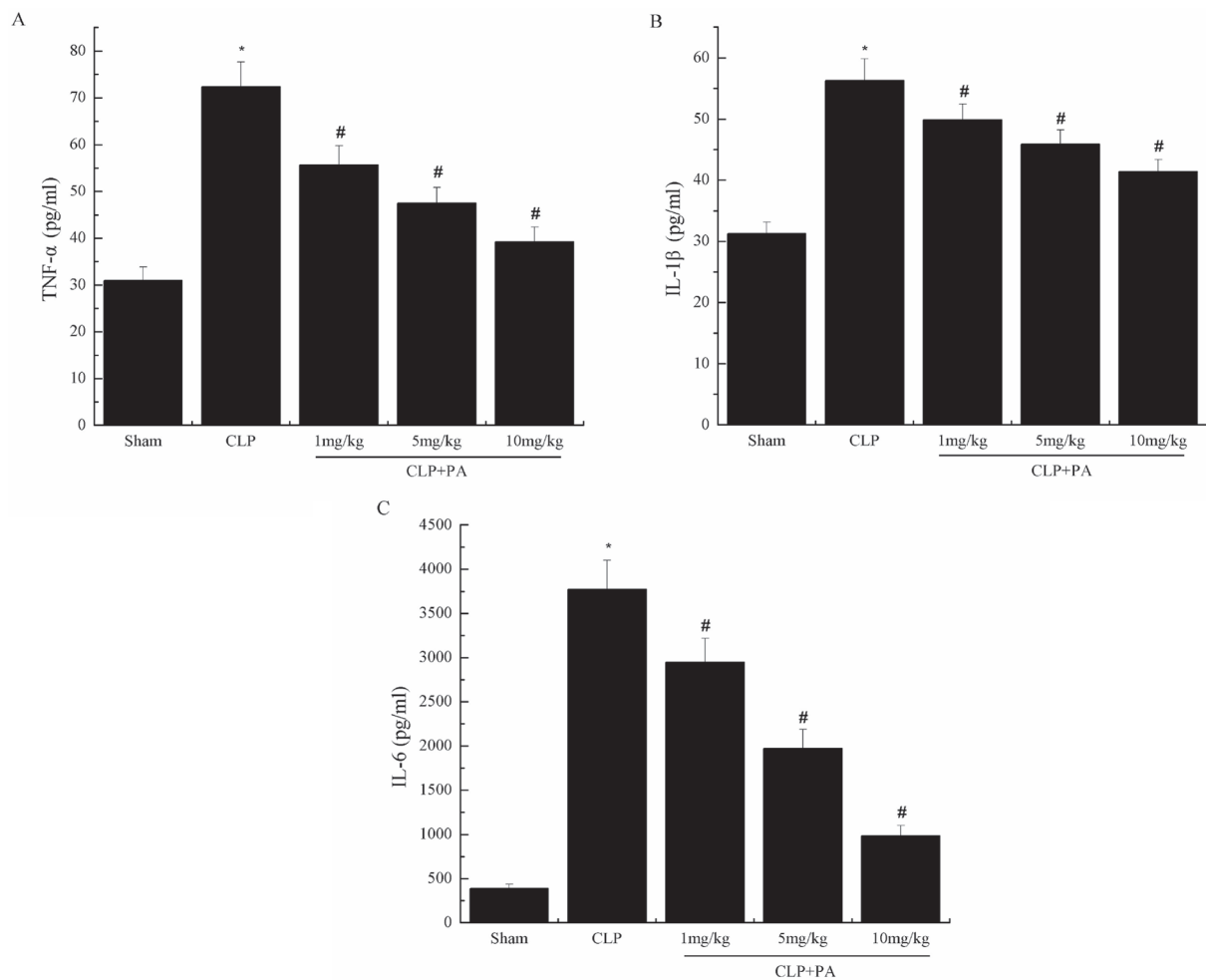


Figure 4. Effect of PA on serum cytokine levels of CLP rats. PA markedly reduces the serum levels of TNF-α (**A**), IL-1β (**B**) and IL-6 (**C**) in the rats underwent CLP surgery. Data were shown as mean ± SD. * $p < 0.05$ vs. sham group, # $p < 0.05$ vs. CLP group.

3771.48 ± 329.25 for IL-6, $p < 0.05$ in each group). However, the treatment of PA suppressed the production of inflammatory cytokines induced by CLP surgery compared with the control group.

Effect of PA on Oxidative Stress Induced by CLP in Lung

To estimate the effect of PA on oxidative stress induced by CLP in lung, we assayed SOD activity, and the contents of MDA and MPO in the lungs of rats. As shown in Figure 5, the activity of SOD was significant declined (from 53.24 ± 5.03 to 24.53 ± 3.17 , $p < 0.05$), along with the noticeable rise of the contents of MDA and MPO (from 8.23 ± 0.29 to 18.35 ± 0.61 for MDA, and from 2.84 ± 0.19 to 14.93 ± 1.02 for MPO, both $p < 0.05$) in CLP group compared with control group. However, the treatment of PA effectively attenuated those changes.

Histological Change

As shown in Figure 6, lung tissues from the sham group showed normal structures and no histopathologic changes. In comparison with the sham group, tissues from the CLP group showed characteristic pathologic changes, including infiltration of inflammatory cells into alveolar space, interstitial edema, and pulmonary congestion. However, those pathologic changes were significantly ameliorated by administration of PA.

Discussion

ALI/ARDS associated with sepsis is a major factor affecting the development and prognosis of critically ill patients in intensive care units. So, it is important to establish a sepsis-induced ALI

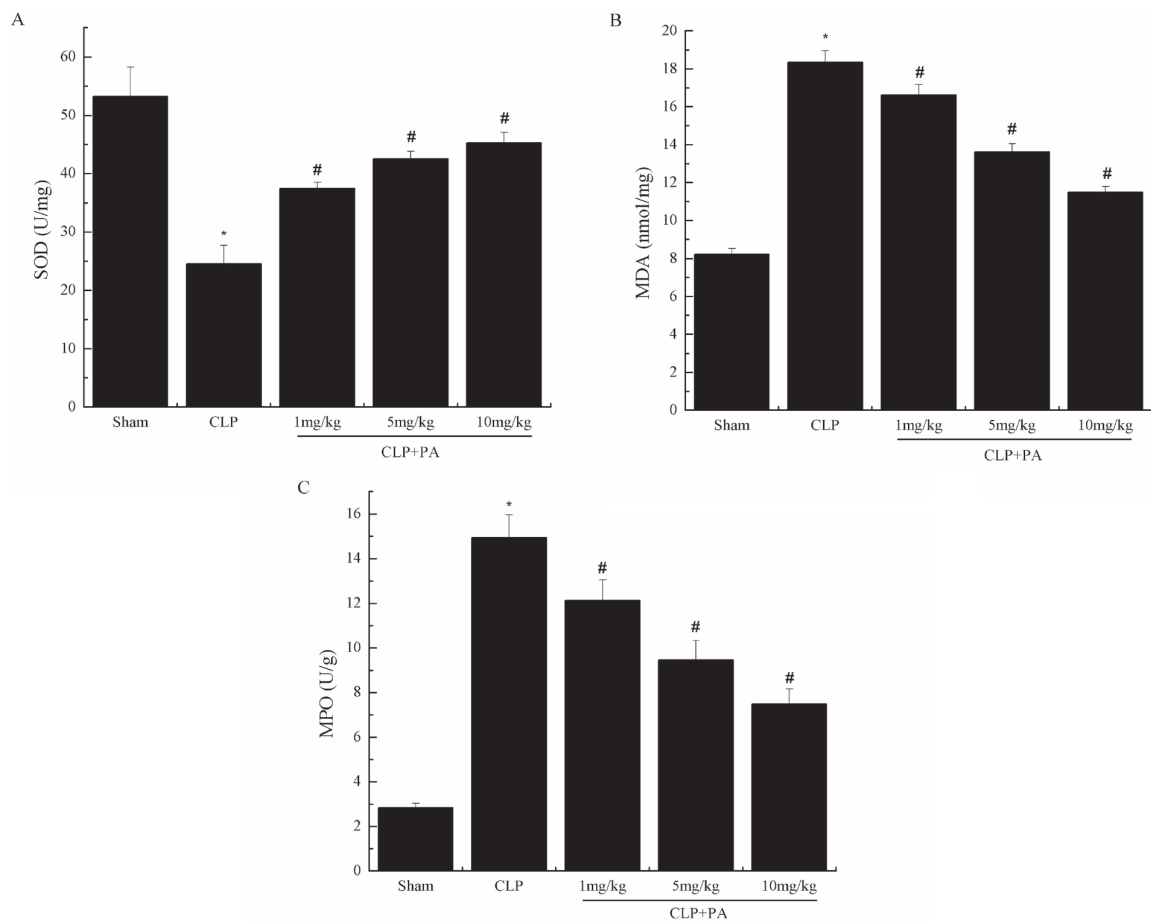


Figure 5. Effect of PA on oxidative stress induced by CLP. PA notably increases the SOD activity **(A)** suppressed by CLP, and reduces the MDA **(B)** and MPO **(C)** contents elicited by CLP. Data were shown as mean $\pm$ SD. * $p < 0.05$ vs. sham group, # $p < 0.05$ vs. CLP group.

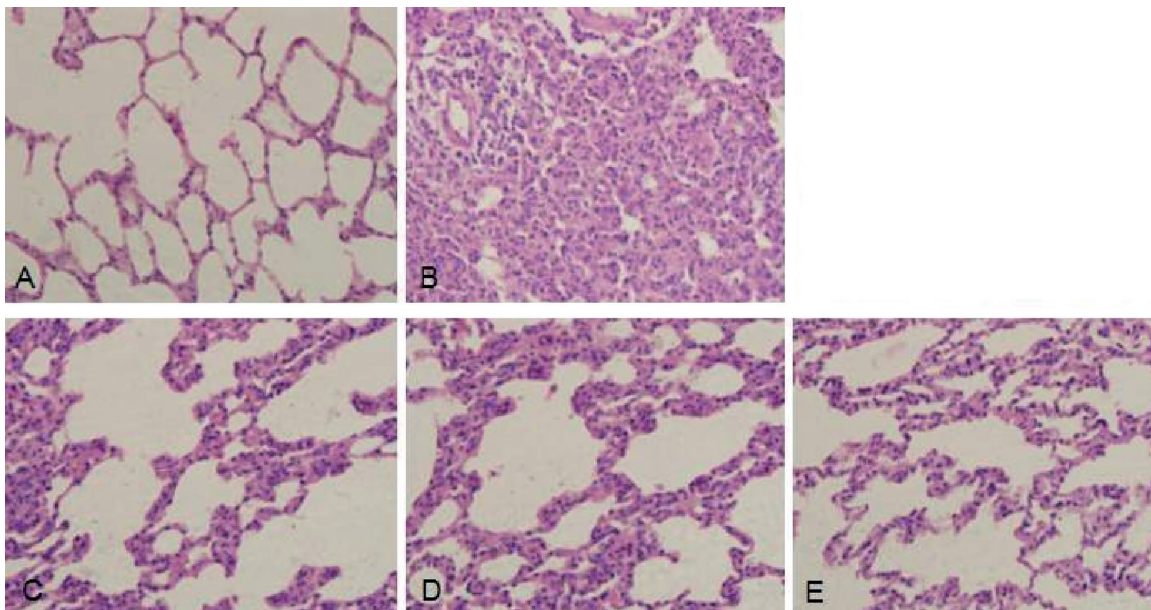


Figure 6. PA ameliorates CLP-induced histologic changes in the lung. **(A)** sham group; **(B)** CLP group; **(C)** CLP + PA 1 mg/kg group; **(D)** CLP + PA 5 mg/kg group; **(E)** CLP + PA 10 mg/kg group (He $\times$ 200).

model to understand the pathogenesis of sepsis and investigate potential therapies. As such, we employed the CLP model, which most closely resembles the pathophysiology of human sepsis¹⁵. In the present study, we used the CLP-induced septic rats to assess the protective effects of PA against septic ALI. The results showed that PA could alleviate sepsis-induced pulmonary edema, gas exchange function, inflammatory responses, and oxidative stress in lungs of ALI induced by CLP, and improved the survival of CLP rats. These findings demonstrated that PA offers a protective role against CLP-induced ALI. Pulmonary interstitial edema and alveolar damage are the characteristics of ALI¹⁶. The present work reports that PA significantly reduced the lung W/D weight ratio induced by CLP challenge. Histologic analysis of lung tissue in rats with CLP-induced ALI presented infiltration of inflammatory cells into alveolar space, interstitial edema, and pulmonary congestion, which was attenuated by PA. A further study revealed that PA significantly ameliorated the low PaO₂ and high PaCO₂ saturation in blood induced by CLP. These findings indicated that PA could prevent the development of pulmonary edema and improve the function of pulmonary gas exchange. Inflammatory cells infiltration into the lung is considered a critical characteristic in the pathogenesis of ALI/ARDS¹⁷. Proinflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, are considered pivotal mediators for sepsis-induced lung injury^{18,19}. In this study, we examined the serum levels of TNF- α , IL-1 β , and IL-6. The data indicated that PA could exert an anti-inflammatory effect in a rat model of sepsis by inhibiting proinflammatory cytokine expression (TNF- α , IL-1 β , and IL-6), which suggests that PA could reduce the lung damage in CLP-induced ALI through inhibiting the inflammatory response. MPO is a major ingredient of neutrophil cytoplasmic granules, its activity in lung tissue is evaluated as an index of pulmonary neutrophil accumulation²⁰. In the present study, animals exposed to CLP challenge had a massive inflammatory cell infiltration into lung tissue, and MPO activity in the CLP group was significantly elevated. However, PA reversed these changes induced by CLP. To further evaluate the therapeutic effect of PA on CLP-induced ALI, we determined the oxidative stress in the lung tissue. During the inflammatory response, overzealous activated neutrophils release extravagant reactive oxygen species (ROS). In normal conditions, there is a balance between the production of oxi-

dants and the protective activities of antioxidants. MDA is the breakdown product of polyunsaturated fatty acids by oxidation, and SOD is the only antioxidant enzyme that scavenges superoxide^{21,22}. In our study, PA caused a remarkable reduction in the MDA content and a significant increase in SOD activity in lungs of CLP rats. The results revealed that the protective effects of PA on CLP-induced ALI may be attributed to the inhibition of oxidative stress in the lung.

Conclusions

Taken together, our study demonstrated that PA has a protective effect on the CLP-induced ALI in septic rats. Thus, PA can be developed as a novel treatment for CLP-induced ALI.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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