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Original Research Article

Physalin A alleviates inflammation and oxidative stress in mouse infantile pneumonia by inhibiting JAK/STAT3 and NF-κB pathways

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Abstract

Purpose: *To investigate the effect of physalin A on infantile pneumonia in mice.*
Methods: *A mouse model of infantile pneumonia was established via intraperitoneal injection of lipopolysaccharide and verified using hematoxylin and eosin staining. Lipopolysaccharide-stimulated mice were then administered physalin A. The total protein content, number of cells, and levels of inflammatory-factor in bronchoalveolar lavage fluid (BALF) were determined. Inflammation and oxidative stress levels in lung tissues were determined by enzyme-linked immunosorbent assay (ELISA).*
Results: *Lipopolysaccharide injection induced shrinking of pulmonary alveoli in mice, but physalin A administration ameliorated the histopathologic damage in lung tissues and significantly reduced the total protein content and number of cells in the BALF of lipopolysaccharide-stimulated mice ($p < 0.001$). Moreover, physalin A also significantly down-regulated the levels of tumor necrosis factor-α, interleukin (IL)-6, IL-18, and IL-1β in BALF and lung tissues of lipopolysaccharide-treated mice ($p < 0.001$). Physalin A attenuated lipopolysaccharide-induced increases in malondialdehyde and myeloperoxidase as well as decreases in superoxide dismutase and glutathione in mouse lung tissues. Additionally, physalin A reduced the levels of p-JAK1, p-STAT3, and p-p65 in lung tissues of lipopolysaccharide-treated mice.*
Conclusion: *Physalin A exerts anti-inflammatory and anti-oxidant effect on lipopolysaccharide-induced lung injury in mice through the inactivation of JAK/STAT3 and NF-κB pathways. However, the effect of Physalin A on inflammation and oxidative stress in lipopolysaccharide-induced A549 cells will need to be investigated in further studies.*

Keywords: *Physalin A, Inflammation, Oxidative stress, Lipopolysaccharide, Lung injury, Infantile pneumonia*

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INTRODUCTION

Infantile pneumonia is caused by *Mycoplasma pneumonia* and commonly occurs in infants [1]. A high fever in children with infantile pneumonia may result in febrile convulsions and even heart or respiratory failure [2]. Although antibiotics and antiviral drugs are used in the treatment of infantile pneumonia with a good prognosis, overuse of antibiotics and serious adverse

reactions caused by antiviral drugs limit the therapeutic efficacy of these strategies [3]. Therefore, developing more effective therapeutic strategies is essential for treating infantile pneumonia.

A previous study showed that *Physalis alkekengi* L. var. *franchetii* attenuated lipopolysaccharide-induced acute lung injury and suppressed inflammatory and oxidant lung injury [4]. Physalin A is the main constituent of traditional Chinese medicine, Physalis Calyx seu Fructus, which has pharmacological properties including antifungal, anti-irritant, anti-inflammatory, and analgesic [5]. Physalin A retarded acetic acid-induced capillary permeability and carrageenan-induced paw edema in mice and reduced lipopolysaccharide-induced secretion of inflammatory factors in RAW 264.7 cells [6]. However, the role of physalin A in infantile pneumonia remains unclear.

Emerging evidence has shown a strong correlation between the severity of pneumonia and the degree of inflammation [7]. Infantile pneumonia is regarded as an acute inflammatory disorder of the lungs [8]. Mycoplasma infection induces pulmonary inflammation and leads to excessive secretion of pro-inflammatory factors, resulting in pulmonary injury in infantile pneumonia [9]. Inhibition of inflammatory injury ameliorated development of infantile pneumonia [8]. Physalin A exerted an anti-inflammatory effect against lipopolysaccharide-induced RAW 264.7 cells [6]. Therefore, physalin A might also reduce lipopolysaccharide-induced secretion of inflammatory factors in mice and attenuate infantile pneumonia.

The aim of this study was to investigate the effects of physalin A on inflammation and oxidative stress in lipopolysaccharide-treated mice, and the underlying mechanism of action in order to develop a novel strategy for the clinical treatment of infantile pneumonia.

EXPERIMENTAL

Animal model

A total of 40 male c57BL/6 mice weighing 4 – 5 g, 1 week old were acquired from the Animal Laboratory of Shandong University (Shandong, China). The mice were randomly assigned to five groups: sham (n = 8), lipopolysaccharide (lipopolysaccharide, n = 8), lipopolysaccharide + 2.5 mg/kg physalin A (n = 8), lipopolysaccharide + 5 mg/kg physalin A (n = 8), and lipopolysaccharide + 10 mg/kg Physalin A (n = 8) with 8 mice in each group. Mice in the sham group were anesthetized with 50 mg/kg pentobarbital sodium (Sigma-Aldrich, St. Louis, MO, USA) via orotracheal intubation using a 20-G intravenous cannula. The mice were then instilled with 30 µL phosphate-buffered saline. Mice in all other groups were instilled with 30 µL of 2 mg/kg lipopolysaccharide in phosphate-buffered saline.

Physalin A (ChemFaces, Wuhan, China) was administered to mice using gastric perfusion once daily for 3 consecutive days. The bronchoalveolar lavage fluid (BALF) and lung tissues were collected 24 h after lipopolysaccharide administration. All animal experiments were approved by the Ethics Committee of the First People's Hospital of Nantong City (Approval no. S20210318-002) and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [10].

Hematoxylin and Eosin staining

Lung tissues were fixed in 4 % paraformaldehyde for 24 h. Paraffin-embedded lung tissues were cut into 5-µm sections, which were dewaxed in xylene, rehydrated in ethanol, and stained with hematoxylin and eosin (Sigma-Aldrich). The slices were observed under light microscopy (Olympus Corporation, Tokyo, Japan).

Bronchoalveolar lavage

Mice were cannulated using 20-G intravenous cannulas after the trachea was exposed and then lavaged with 1 mL phosphate-buffered saline to harvest the BALF. The bronchoalveolar lavage supernatant was collected after centrifugation at 500 *g* for 10 min. The total protein content of BALF was determined using a bicinchoninic acid protein assay (Beyotime, Beijing, China), and the total number of cells in the BALF was calculated using blinded manual cell counting.

Enzyme-linked immunosorbent assay (ELISA)

Lung tissues were lysed in RIPA buffer (Beyotime), and the supernatant was collected after centrifugation at 12,000 *g* for 60 min. Levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-18, and IL-1β levels in the BALF and supernatant of lung tissues were determined via ELISA kits (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China). The levels of malondialdehyde (MDA), myeloperoxidase (MPO), superoxide dismutase (SOD), and glutathione (GSH) in lung tissues were also assessed using ELISA kits.

Western blotting

Lung tissues were lysed in RIPA buffer (Beyotime) to harvest the supernatant. Protein samples in the supernatant of lung tissues were separated using sodium dodecyl-sulfate polyacrylamide gel electrophoresis and electro-transferred onto a polyvinylidene difluoride membranes. The membranes were blocked with 5 % skim milk and probed with the following primary antibodies at 4 ºC overnight: anti-p-JAK1 and anti-JAK1 (1:2000), anti-p-STAT3 and anti-STAT3 (1:3000), anti-p-p65 and anti-p65 (1:4000), anti-p-IκBα and anti-IκBα (1:5000), and anti-β-actin (1:6000). The membranes were incubated with a horseradish peroxidase-labeled secondary antibody (1:5000) and subjected to enhanced chemiluminescence (Beyotime) to detect immunoreactivity. All antibodies were purchased from Abcam (Cambridge, UK).

Statistical analysis

The data are expressed as mean ± standard error of the mean (SEM) and were analyzed with Student's *t*-test or one-way analysis of variance using GraphPad Prism software. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Physalin A attenuated lipopolysaccharide-induced pulmonary injury

Mice were treated with lipopolysaccharide to induce pulmonary injury. Lipopolysaccharide injection produced histopathologic damage in lung tissues of mice, as demonstrated by inflammatory cell infiltration, alveolar hemorrhage, interstitial edema, and alveolar septal thickening (Figure 1 A). However, physalin A administration attenuated the lipopolysaccharide-induced histopathologic damage in lung tissues of mice (Figure 1 A). Physalin A also reduced the total protein content (Figure 1 B), and number of cells (Figure 1 C) in the BALF from lipopolysaccharide-treated mice in a dose-dependent manner.

Physalin A attenuated lipopolysaccharide-induced inflammation

Levels of TNF-α, IL-6, IL-18, and IL-1βin mouse lung tissues (Figure 2 A) and BALF (Figure 2 B) were up-regulated after lipopolysaccharide injection. Physalin A reduced levels of TNF-α, IL-6, IL-18, and IL-1β (Figure 2 A and B) in a dose-dependent manner.

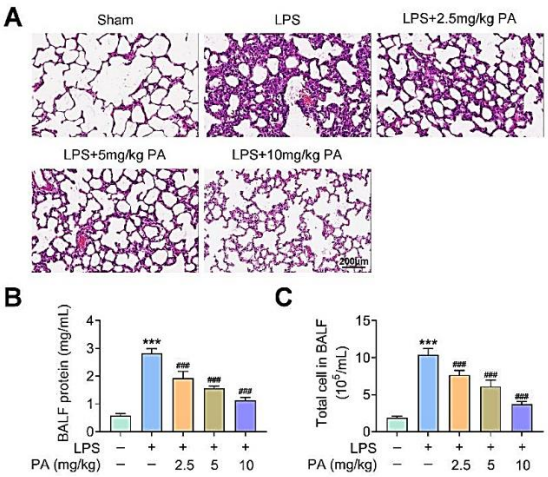

Figure 1: Physalin A attenuated lipopolysaccharide-induced pulmonary injury. (A) Administration of physalin A attenuated lipopolysaccharide-induced histopathologic damage in mouse lung tissues. (B) Physalin A attenuated lipopolysaccharide-induced increase in total protein content in the BALF of mice. (C) Physalin A attenuated lipopolysaccharide-induced increase in total cell number in the BALF of mice. ***$P < 0.001$ vs. sham. $^{###}p < 0.001$ vs. lipopolysaccharide

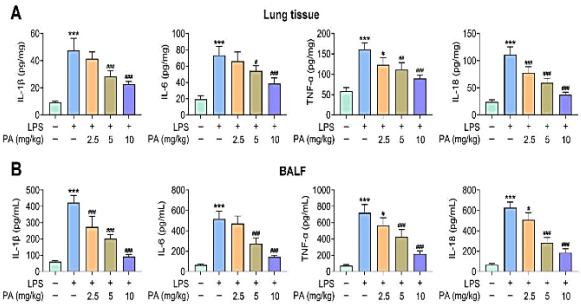

Figure 2: Physalin A attenuated lipopolysaccharide-induced inflammation. (A) Physalin A attenuated the lipopolysaccharide-induced up-regulation of TNF-α, IL-6, IL-18, and IL-1β in mouse lung tissues. (B) Physalin A attenuated the lipopolysaccharide-induced up-regulation of TNF-α, IL-6, IL-18, and IL-1β in the BALF of mice. ***$P < 0.001$ vs. sham; $^{#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$ vs. Lipopolysaccharide

Physalin A attenuated lipopolysaccharide-induced oxidative stress

The level of MDA was increased in lung tissues of lipopolysaccharide-treated mice (Figure 3). However, physalin A reduced MDA in the lung tissues of lipopolysaccharide-treated mice (Figure 3). Lipopolysaccharide-induced up-regulation of MPO and down-regulation of SOD and GSH in mouse lung tissues (Figure 3). However, it also enhanced SOD and GSH and reduced MPO levels in lipopolysaccharide-treated mice (Figure 3).

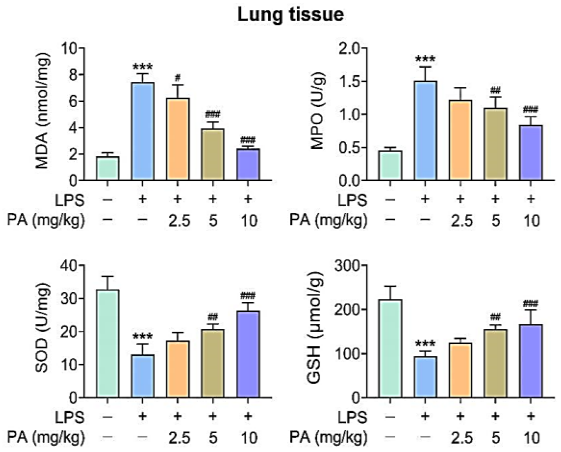

Figure 3: Physalin A attenuated lipopolysaccharide-induced oxidative stress. Physalin A attenuated the lipopolysaccharide-induced down-regulation of SOD and GSH and increase of MDA and MPO in mouse lung tissues. ***P < 0.001, vs. sham; $^{\#}p$ < 0.05, $^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001 vs. lipopolysaccharide

Physalin A inhibited lipopolysaccharide-induced activation of JAK/STAT3 and NF-κB pathways

Lipopolysaccharide-induced up-regulation of p-JAK1 and p-STAT3 in mouse lung tissues (Figure 4 A). Physalin A reduced p-JAK1 and p-STAT3 in lipopolysaccharide-treated mice (Figure 4 A). Moreover, Physalin A attenuated the lipopolysaccharide-induced increases in p-p65 and p-IκBα in lung tissues (Figure 4 B).

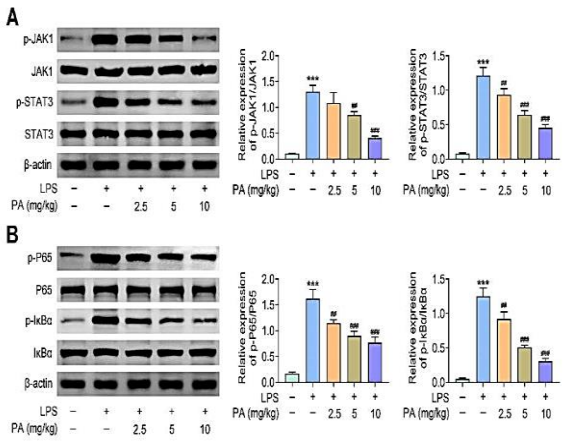

Figure 4: Physalin A inhibited lipopolysaccharide-induced activation of the JAK/STAT3 and NF-κB pathways. (A) Physalin A attenuated the lipopolysaccharide-induced up-regulation of p-JAK1 and p-STAT3 in mouse lung tissues. (B) Physalin A attenuated lipopolysaccharide-induced up-regulation of p-p65 and p-IκBα in mouse lung tissues. ***P < 0.001 vs. sham; $^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001 vs. lipopolysaccharide

DISCUSSION

This study found that a novel agent, Physalin A, protected mice against lipopolysaccharide-induced acute lung injury. Lipopolysaccharide induces pulmonary inflammation and is widely used to establish acute lung injury models [11]. The results of this study showed that lipopolysaccharide-induced shrinking of pulmonary alveoli resulted in pulmonary injury in mice. Moreover, it has been reported that lipopolysaccharide up-regulates the total protein content and number of cells in the BALF of newborn mice during the development of infantile pneumonia [12]. This study also showed up-regulation of the total protein content and number of cells in the BALF of lipopolysaccharide-treated mice. Physalin A ameliorated histopathologic damage in lung tissues of lipopolysaccharide-induced mice and reduced the total protein content and number of cells in the BALF.

Anti-inflammatory agents have been considered promising strategies to treat infantile pneumonia [13]. The NF-κB signaling, which is essential for the secretion of pro-inflammatory factors, was activated in lipopolysaccharide-induced mice, and NF-κB knockdown suppressed lipopoly-saccharide-induced pulmonary inflammation in mice [13]. The results of this study demonstrated that lipopolysaccharide-induced up-regulation of TNF-α, IL-6, IL-18, and IL-1β in the lung tissues and BALF of mice. The expression levels of p-p65 and p-IκBα were also increased in mice after lipopolysaccharide injection. A previous study showed that physalin A exerted anti-inflammatory effects through the inactivation of NF-κB signaling [14]. Physalin A also reduced secretion of IL-1β, IL-6, and TNF-αin lipopolysaccharide-induced RAW 264.7 cells through down-regulation of NF-κB [15]. In this study, Physalin A also reduced levels of TNF-α, IL-6, IL-18, and IL-1βin the lung tissues and BALF of lipopolysaccharide-induced mice and down-regulated p-p65 and p-IκBα protein expression. Therefore, Physalin A might exert an anti-inflammatory effect against infantile pneumonia through the inactivation of NF-κB signaling.

Macrophages and polymorphonuclear neutrophils reduce lung infection caused by *Mycoplasma pneumoniae* through accumulation of reactive oxygen species. However, excessive reactive oxygen species might increase oxidative stress in lungs and lead to direct pulmonary injury [16]. Oxidative stress also results in a local inflammatory response during the development of pneumonia [16]. Therefore, oxidative stress is involved in the pathogenesis of infantile pneumonia. Lipopolysaccharide-induced

increase of MDA and MPO and down-regulation of SOD and GSH in lung tissues of mice, thereby promoting oxidative stress. Physalin A inhibited MDA production and enhanced levels of the anti-oxidant factors SOD, catalase, and glutathione peroxidase in lipopolysaccharide-treated RAW 264.7 cells [15]. Physalin A enhanced SOD and GSH and reduced MDA and MPO in lipopolysaccharide-induced mice, thereby exerting anti-oxidant effects against infantile pneumonia.

The JAK/STAT pathway has been reported to be important for regulation of cytokines and growth factors [17]. Activation of the JAK/STAT pathway contributes to inflammatory damage and oxidative stress in schistosome-induced liver injury [17]. Moreover, STAT3 was activated in alveolar macrophages during lipopolysaccharide-induced acute lung injury, and suppression of STAT3 signaling alleviated lung injury in lipopolysaccharide-induced rats [18]. Physalin A inhibited activation of JAK/STAT3 signaling and suppressed proliferation and metastasis of non-small cell lung cancer cells [19]. This study revealed that physalin A reduced levels of p-JAK1 and p-STAT3 in lung tissues of lipopolysaccharide-induced mice. Therefore, physalin A exhibited anti-inflammatory and anti-oxidant effects against infantile pneumonia through inactivation of JAK/STAT3 signaling.

CONCLUSION

Physalin A protects mice against lipopolysaccharide-induced acute lung injury through suppression of inflammatory responses and oxidative stress. It also suppresses the activation of JAK/STAT3 and NF-κB pathways and attenuates infantile pneumonia. However, the effect of Physalin A on inflammation and oxidative stress in lipopolysaccharide-induced A549 cells will need to be investigated in further studies.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Zhenhua Ling and Jinhua Zhao designed and carried out the study, supervised data collection, analyzed and interpreted the data, prepared the manuscript for publication, and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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