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Original Research Article

Pristimerin attenuates sepsis-induced lung injury by regulating nuclear factor kappaB/high-mobility group box 1 pathway

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Abstract

Purpose: *To determine the effect of pristimerin on sepsis-induced lung injury, and the underlying mechanism of action.*
Methods: *Lung injury was established in mice via induction of sepsis through cecal ligation and puncture (CLP). The effect of pristimerin was evaluated based on lung wet/dry weight and PaO_2/FiO_2 ratios. Lung tissue was subjected to immunohistochemical and histopathological analyses, as well as Western blotting. Furthermore, the serum levels of inflammatory mediators were determined.*
Results: *Pristimerin reversed the altered lung wet/dry weight ratio and PaO_2/FiO_2 ratio in the lung, and also reduced lung injury score, relative to CLP group ($p < 0.05$). Moreover, it suppressed nucleocytoplasmic translocation of high mobility group protein B1 (HMGB1) in lung tissue. Serum levels of inflammatory mediators and expression levels of inducible nitric oxide synthase and nuclear factor-kappaB p65 were significantly reduced by pristimerin ($p < 0.05$).*
Conclusion: *Pristimerin ameliorates sepsis-induced lung injury by inhibiting HMGB1/NF-κB. Thus, this compound has a potential for clinical application in the management of lung injury.*

Keywords: *Pristimerin, Sepsis, Lung injury, Inflammatory mediators, HMGB1*

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INTRODUCTION

Sepsis, a systemic inflammation due to infection, is associated with multiple organ dysfunction, and it is a leading cause of death in intensive care units [1]. Globally, about 19 million patients suffer from sepsis annually. One of the major complications of sepsis is acute lung injury [2]. The prognosis of patients with sepsis-induced lung injury is very poor, and high mortality often occurs. Therefore, there is need for therapeutic interventions for lung injury [3]. In sepsis, inflammation is mediated by altered expression of high-mobility group box 1 (HMGB1) which is also involved in the pathogenesis of lung injury [4]. High-mobility group box 1 (HMGB1) which is secreted by monocytes and macrophages, is a late mediator of inflammation in sepsis [5]. It induces the production of proinflammatory cytokines by activating the nuclear factor-kappaB

(NF-□B) pathway [6]. Studies have shown that NF-κB participates in the pathogenesis of lung injury by increasing the expressions of cytokines that contribute to systemic inflammation by stimulating the acetylation of the p65 subunit [7].

Alternative medicines for the management of chronic disorders have continued to receive a lot of attention. Several natural medicines have shown potential for the treatment of lung injury. Pristimerin is a triterpenoid isolated from *Celastraceae* [8]. It is used in traditional Chinese medicine for treating inflammatory and autoimmune disorders and tumours [9]. Pristimerin exerts strong antioxidant effects by inhibiting inducible nitric oxide synthase (iNOS), thereby reducing nitric oxide (NO) level [10]. This results in anti-inflammatory and hepatoprotective effects [11]. Moreover, pristimerin inhibits the inflammatory cascade, and reduces NF-κB levels [12]. The present study determined the effect of pristimerin on sepsis-induced lung injury in mice.

EXPERIMENTAL

Animals

Male albino mice weighing 20 – 25 g, were purchased from Shanghai Medical College, China. The mice were acclimatised under a 12-h day/12-h night light cycle with 60 ± 5% humidity at 24 ± 3°C, according to the guidelines of Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) [13]. All experimental procedures involving animals were approved by the Institutional Animal Ethics Committee of Guizhou Provincial People's Hospital, China (IAEC/GPPH/2017/04).

Lung injury was induced via sepsis, which was in turn induced by cecal ligation and puncture (CLP). The mice were fasted overnight and anesthetised with pentobarbital at a dose of 30 mg/kg intraperitoneally, i.p. The cecum was subjected to laparotomy. An 18-gauge needle was used to puncture the region below the ileocecal valve twice, to ligate the cecum. The abdominal cavity was closed after returning the caecum. In the sham-operated group, only laparotomy was performed. The mice were divided into control, CLP, and two pristimerin groups given the drug at a dose of 10 or 100 mg/kg i.p, 30 min after surgery.

Determination of PaO_2/FiO_2 ratio

The mice were anesthetised 1 day after CLP, and arterial blood samples were subjected to PaO_2 analysis. The oxygenation index was expressed in terms of PaO_2/FiO_2 ratio.

Histopathological examination of lung tissue

The mice were euthanised, and their lungs were excised and fixed in formalin (10 %) for 3 days. Sections of the pulmonary lobes were seeded in paraffin and sectioned to 3-µm thick slices using a microtome. The sections were stained with haematoxylin and eosin. Infiltration of inflammatory cells, necrosis, interstitial oedema and haemorrhage were determined using a scale ranging from 0–3.

Immunohistochemical analysis

The 3-µm thick sections of lung tissue were blocked for 10 min in 3 % H_2O_2. An anti-HMGB1 (1:1000) antibody was then added, followed by visualisation with 3,3′-diaminobenzidine and counter-staining with Mayer's haematoxylin.

Determination of inflammatory mediators

The serum levels of HMGB1, interleukin (IL)-6, and tumour necrosis factor (TNF)-α were determined using enzyme-linked immunosorbent assay according to the manufacturer's instructions. Serum nitrite concentration was determined using a nitrite/nitrate colorimetric assay kit.

Western blotting

Total protein from lung tissue homogenate was resolved with 8 % sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and transferred to a nitrocellulose membrane. The membrane was blocked in H_2O_2 and incubated for 1 h with primary antibodies against α-tubulin, iNOS, NF-κB p65, and β-actin. Thereafter, the membrane was washed twice with phosphate-buffered saline and incubated for 30 min with horseradish peroxidase-conjugated goat anti-mouse IgG. A chemiluminescence kit was used to develop the bands, the intensity of which was quantified with densitometric analysis.

Statistical analysis

Data are presented as mean ± standard deviation (SD) (n = 6). Statistical analysis was performed using one-way analysis of variance with Dunnett's *post hoc* test. All statistical analyses were performed using Prism software (ver. 6.1; GraphPad Software, Inc., La Jolla, CA, USA). Values of $p < 0.05$ were considered indicative of statistical significance.

RESULTS

Pristimerin reduced lung wet/dry weight ratio, but increased PaO_2/FiO_2 ratio

The lung wet/dry weight ratio was increased, while the PaO_2/FiO_2ratio was decreased in the CLP group, when compared to the control group (Figure 1). The lung wet/dry weight ratio was significantly reduced, while the PaO_2/FiO_2 ratio was significantly increased ($p < 0.01$) by pristimerin.

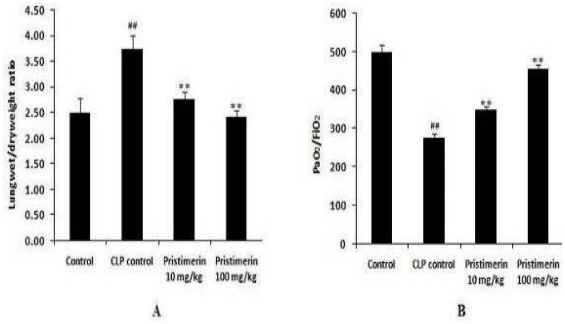

Figure 1: Effect of pristimerin on lung wet/dry weight and PaO_2/FiO_2 ratios in mice with sepsis-induced lung injury. Data are mean ± SD (n = 6); $^{\#\#}p < 0.01$ *vs.* control group; $^{**}p < 0.01$ *vs*. cecal ligation and puncture (CLP) group

Pristimerin lowered sepsis-induced increase in lung injury score

Lung histology was significantly altered in the CLP group, when compared to the control group (Figure 2). Lung injury scores (haemorrhage, necrosis, oedema, and neutrophil infiltration) were significantly increased in the CLP group, relative to the control group. However, these increases were attenuated by pristimerin.

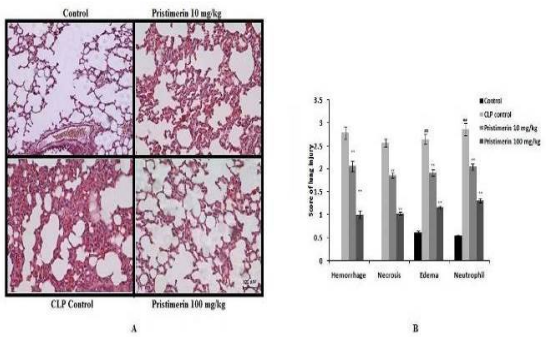

Figure 2: Effect of pristimerin on lung histopathology in mice with sepsis-induced lung injury. A: Haematoxylin and eosin staining (x100); B: lung injury scores. Data are mean ± SD (n = 6); $^{\#\#}p < 0.01$ *vs.* control group; $^{**}p < 0.01$ *vs*. CLP group

Pristimerin reduced the expression of HMGB1

Nucleocytoplasmic translocation of HMGB1 in lung tissue was suppressed by pristimerin, relative to the CLP group (Figure 3).

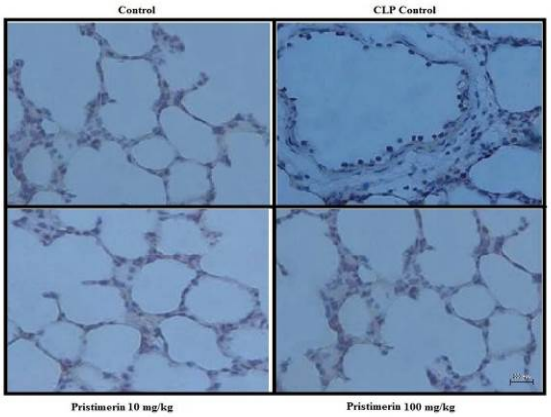

Figure 3: Effect of pristimerin on the expression of HMGB1 in the lung tissue of mice with sepsis-induced lung injury: results of immunohistochemical analysis

Pristimerin modulated the levels of inflammatory mediators

Figure 4 shows that the serum levels of HMGB1, TNF-α, IL-6, and

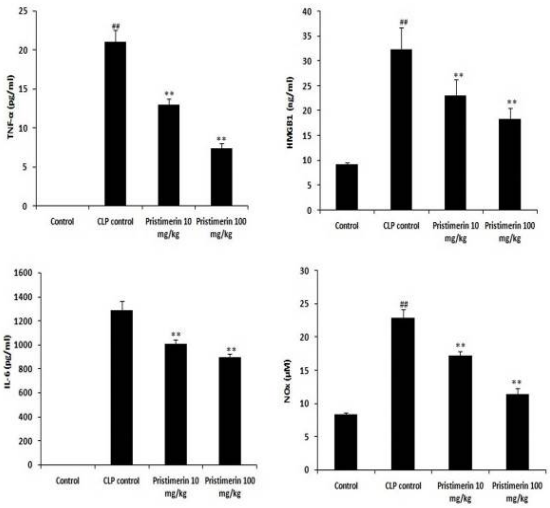

Figure 4: Effect of pristimerin on the serum levels of inflammatory mediators in mice with sepsis-induced lung injury. Data are mean ± SD (n = 6); $^{\#\#}p < 0.01$ *vs*. control group; $^{**}p < 0.01$ *vs*. CLP group

Pristimerin reduced the expressions of iNOS and NF-κB p65

The expressions of iNOS and NF-κB p65 were significantly increased in the lung tissue of the

CLP group, relative to the control group ($p < 0.01$). However, this effect was significantly reversed by pristimerin ($p < 0.01$; Figure 5).

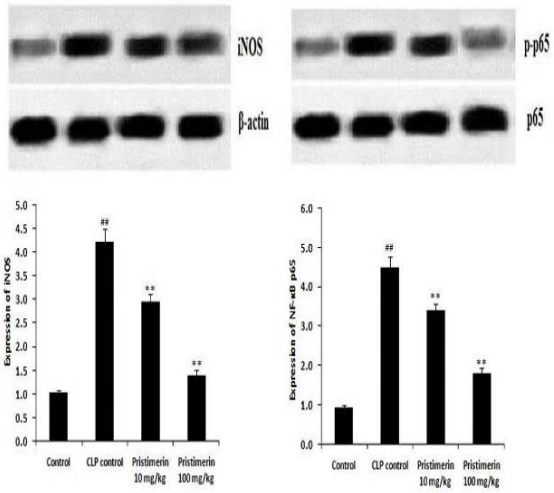

Figure 5: Effect of pristimerin on the expressions of inducible nitric oxide synthase (iNOS) and nuclear factor kappa B (NF-κB) p65 in lung tissue of mice with sepsis-induced lung injury. Data are mean ± SD (n = 6); $^{\#\#}p < 0.01$ *vs*. control group; $^{**}p < 0.01$ *vs*. CLP group

DISCUSSION

The present study evaluated the protective effect of pristimerin against sepsis-induced lung injury by determining lung wet/dry weight ratio, PaO_2/FiO_2 ratio, and serum levels of inflammatory mediators.

The expressions of iNOS and inflammatory cytokines are induced by NF-κB. Inhibitor of kappaB is translocated to the nucleus in response to various stimuli, where it induces inflammation and cell adhesion and proliferation [13]. It is known that NF-κB regulates innate immune response [14]. In this study, pristimerin decreased serum levels of iNOS, TNF-α, and IL-6, and significantly decreased NF-κB level in the lung tissue of mice with sepsis-induced lung injury.

Pristimerin attenuated haemorrhage, necrosis, oedema and neutrophil infiltration in mice with sepsis-induced lung injury, in agreement with a previous report [15]. Studies have shown that HMGB1 acts as a late mediator of inflammation in sepsis-induced lung injury [16]. In the present study, pristimerin suppressed the nucleocytoplasmic translocation of HMGB1 in the lung tissue of mice with sepsis-induced lung injury.

CONCLUSION

Pristimerin ameliorates sepsis-induced lung injury in rats by inhibiting HMGB1 and NF-κB. Therefore, pristimerin may be useful clinically for the management of lung injury. However further investigations are required to ascertain this.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is 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. XW designed and supervised the protocol and prepared the manuscript, LH and PL performed the study protocol and statistical analysis of presented work.

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