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Treatment of acute lung injury in mice using Bai-Ri-Ke syrup

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Competing interests

The authors declare no conflicts of interest.

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Abbreviations

BRK, Bai-Ri-Ke syrup; ALI, acute lung injury; DXM, dexamethasone; BALF, bronchoalveolar lavage fluid.

Peer review information

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Ethical approval

Ethical approval was obtained from the Tianjin Second People's Hospital, Number [2019]46.

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Abstract

Objective: This study aimed to examine the therapeutic effects of Bai-Ri-Ke syrup (BRK) on mice with acute lung injury (ALI). **Methods:** Fifty male C57BL/6 mice were equally divided into the control group, model group, dexamethasone (DXM) group, Bai-Ri-Ke syrup (BRK) low-group, and BRK-high group, with six mice per group. An intratracheal injection of 5 mg/kg POLY(I:C) was used to construct an ALI mouse model. After a successful model construction, the mice in the DXM group were given [0.2 mg/10 g·d] dexamethasone sodium phosphate injection (1 mL:2 mg) on the following day via intraperitoneal injection. The mice in the BRK-low group were given 0.015 mL APS everyday by gavage, and the mice in the BRK-high group were given 0.030 mL APS everyday by gavage for three days. The wet to dry weight (W/D) ratio of the lungs was observed every day. Bronchoalveolar lavage fluids (BALFs) were collected from the left lungs to measure the BALF protein level and neutrophil count after 72 h of treatment. The IL-6, IL-1β, and TNF-α levels in BALF were also measured. HE staining was done to observe the histopathological changes in the lungs. **Results**: The ALI mice in the BRK-high group had significantly increased W/D ($P < 0.01$). ELISA results showed that the DXM group and BRK-high group had significantly decreased BALF protein content ($P < 0.01$), neutrophil count ($P < 0.01$), and IL-6, IL-1β, and TNF-α levels ($P < 0.01$). Hematoxylin and eosin (H&E) results showed that the DXM group and BRK-high group had alleviated alveolar tissue injury, edema, bleeding, and inflammation. **Conclusions:** The BRK can decrease the W/D, BALF protein content, neutrophil count, and TNF-α, IL-1β, and IL-6 levels and alleviate the histopathological changes in the lungs of ALI mice.

Keywords: pertussis acute lung injury; inflammation bronchoalveolar lavage fluids

Introduction

Acute lung injury (ALI) is a clinical syndrome caused by characteristic pathological changes in lung tissues due to severe infection, shock, and trauma, and bacterial infection is its most common etiology [1]. In clinical practice, ALI is a syndrome that is characterized by non-cardiogenic pulmonary edema and refractory hypoxemia. Severe ALI has a mortality rate of 30–40%. If there is also sepsis, the mortality rate of ALI is as high as 90%. Reports showed that the mortality rate of ALI accompanied with more than four organ failures is 100% [2, 3]. Therefore, it is extremely important and urgent to examine the effective measures and drugs for ALI treatment.

A Bai-Ri-Ke syrup (BRK) is a pure traditional Chinese medicine formula that is used to treat pediatric pertussis. It has heat-dispelling, lung-cleansing, adversity-calming, and phlegm-reducing effects. It is composed of *Stemona japonica*, *Morus alba* L., *Belamcanda chinensis*, *Prunus persica*, *Platycodon grandiflorus*, almonds, and *Andrographis paniculata* and was formulated based on traditional Chinese medicine syndrome identification combined with clinical practice. It is also fast-acting and has a clear therapeutic effect, high response rate, no toxic side effects, and is not easy to recur [4]. The objective of this study was to examine the therapeutic effects of BRK for the treatment of mice with ALI and to provide a reference for its clinical application.

Materials and methods

Reagents

A BRK (Jin Drug Preparation No. Z20190009000) was purchased from the Second Hospital of Tianjin. ELISA kits were purchased from Nanjing Jiancheng Bioengineering Institute. POLY(I:C) (24939-03-5) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. A dexamethasone sodium phosphate injection (NMPA approval no. H22022889) was purchased from Jilin Aodong Pharmaceutical Industry Group Yanji Co., Ltd.

Construction of an ALI mouse model

Male C57BL/6 mice weighing 20 ± 2 g were selected. These were animal grade: SPF grade, housed at room temperature (22 ± 2) ℃, and given sterile feed and water. The mice were anesthetized, and the trachea was surgically exposed. Then, 5 mg/kg POLY(I:C) (dissolved in 50 μL PBS) was administered by intratracheal injection, and the wound was sutured. Ethical approval was obtained from the Tianjin Second People's Hospital, Number [2019]46.

Experiment grouping and dosing regimen

Thirty male C57BL/6 mice were equally divided into the control group, model group, DXM group, BRK-low group, and BRK-high group, with six mice per group. After a routine housing for one week, an ALI model was constructed. After a successful model construction, the mice in the model group were given 0.2 mL of physiological saline everyday via intragastric administration. The mice in the DXM group were given a [0.2 mg/(10 g·d)] dexamethasone sodium phosphate injection (1 mL:2 mg) on alternate days via intraperitoneal injection for a total of two doses. The mice in the BRK-low group were given 0.015 mL of BRK everyday via intragastric administration, while the mice in the BRK-high group were given 0.030 mL of BRK everyday via intragastric administration. The dose of administered BRK was obtained by converting the daily dose for humans to that for animals.

Wet/dry weight ratio of the lung

After an open-chest surgery, the left lungs were harvested from the mice. A filter paper was used to absorb the surface liquid of the lungs, and the wet weight was measured using an electronic balance. After weighing, the lungs were dried at 80 ℃ for 48 h; then, the dry weight was measured. The wet/dry weight ratio (W/D) of the lungs was calculated.

Collection of bronchoalveolar lavage fluid (BALF) and protein content measurement

For anesthesia, 0.01 mL/g of 1% nembutal was administered via intraperitoneal injection. Tracheal intubation was performed, and a hemostatic clamp was used to clamp the right bronchus. PBS was used to flush the left lungs twice with 0.3 mL each time, and aspiration was repeated thrice. PBS was centrifuged at 4 ℃ and 5,000 r/min for 10 min. The supernatant was collected and stored in a freezer at –80 ℃. The pellet was resuspended using 200 μL of PBS to obtain the cell suspension. Bicinchoninic acid (BCA) was used to measure the protein content. An assay was performed based on the manufacturer's instructions in the BCA kit.

BALF Neutrophil count measurement

Meanwhile, 1 μL of 0.4% trypan blue was added to 9 μL of BALF cell suspension, and a hemocytometer was used to measure the cell viability within 3 min. The test results showed that the cell viability was ≥ 95%. Smear preparation was carried out, followed by Giemsa staining. After drying, one field was randomly selected under a 400× optical microscope, and the proportion of neutrophils in 200 cells was counted. This was multiplied by the total number of white blood cells to obtain the neutrophil count.

ELISA detection of cytokine concentration

ELISA was used to measure the concentrations of inflammatory cytokines IL-6, IL-1β, and TNF-α in BALF. This was carried out based on the manufacturer's instructions.

Hematoxylin and eosin (H&E) staining of the lungs

The upper lobe of the left lung was harvested and fixed in 10% paraformaldehyde. This was followed by a low-temperature paraffin embedding, sectioning, and H&E staining. Pathological changes in lung tissues were observed under an optical microscope.

Results

Effects of BRK on lung W/D ratio in ALI mice

Tissue exudation and edema of lung tissues were examined by weighing the lungs of ALI mice after different treatments, and the lung W/D ratio was calculated. The results showed that the W/D ratio was significantly increased in ALI mice ($P < 0.01$) with increased lung water content compared to that in the normal group, which demonstrated that the exudation and edema of lung tissues were highly significant. After the pre-treatment with different doses of BRK or dexamethasone, the W/D ratio was significantly decreased in ALI mice ($P < 0.01$), suggesting that the BRK syrup alleviates the exudation and edema of lung tissues (Figure 1).

Effects of BRK on BALF protein content and neutrophil count in ALI mice

BALF was collected to measure its protein content and neutrophil count. Compared to the normal group, the BALF protein content and neutrophil count were significantly increased in ALI mice ($P < 0.01$). Compared to the ALI mice, the BALF protein content and neutrophil count were significantly decreased in the BRK-high group and DXM group ($P < 0.01$), showing that the BRK has some anti-inflammatory effects (Figure 2, Figure 3).

Effects of BRK on cytokine concentrations in ALI mice

ELISA was used to measure the concentration of inflammatory cytokines IL-6, IL-1β, and TNF-α in BALF. The results showed that IL-6, IL-1β, and TNF-α concentrations were significantly increased in BALF from ALI mice ($P < 0.01$). The BALF cytokine concentrations were significantly decreased in the DXM group, BRK-low group, and BRK-high group ($P < 0.01$) in a dose-dependent manner. As the dosing concentration increased, the BRK exhibited inhibitory effects on inflammatory cytokines to a certain extent (Figure 4).

Effects of BRK on lung histopathological changes in ALI mice

Pathological changes in lung tissues were observed under an optical

microscope. Under the optical microscope (40×), normal alveolar septum, absence of pulmonary interstitial edema, and absence of significant inflammatory cell exudation were seen in the normal group. However, severe alveolar destruction, alveolar space fusion, dilation and collapse of alveolar septum, interstitial edema, and infiltration by a considerable number of inflammatory cells were seen in ALI mice. A clear alveolar structure, slightly thickened alveolar wall, and trace amounts of inflammatory cell infiltration were observed in the DXM, BRK-low group, and BRK-high group. These results showed that the BRK had a significant therapeutic effect on ALI mice.

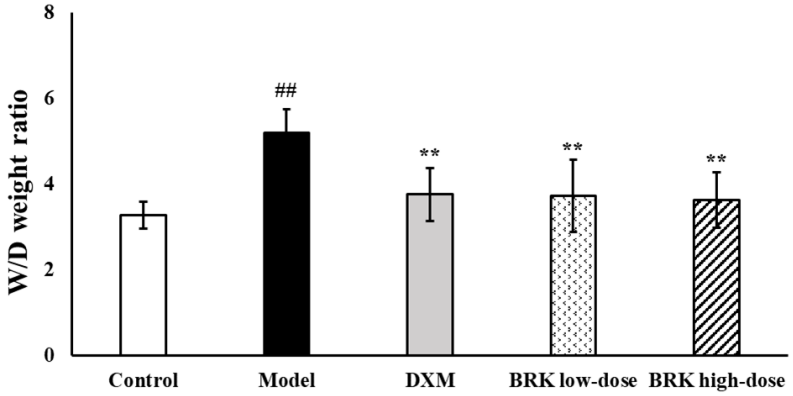

Figure 1 BRK treatment decreased the W/D ratio in Poly(I:C)-induced acute lung injury mice. Control, Model, DXM, BRK low-dose and BRK high-dose groups (n = 6 per group). ##: $P < 0.01$ compared with Control group; **: $P < 0.01$ compared with Model group.

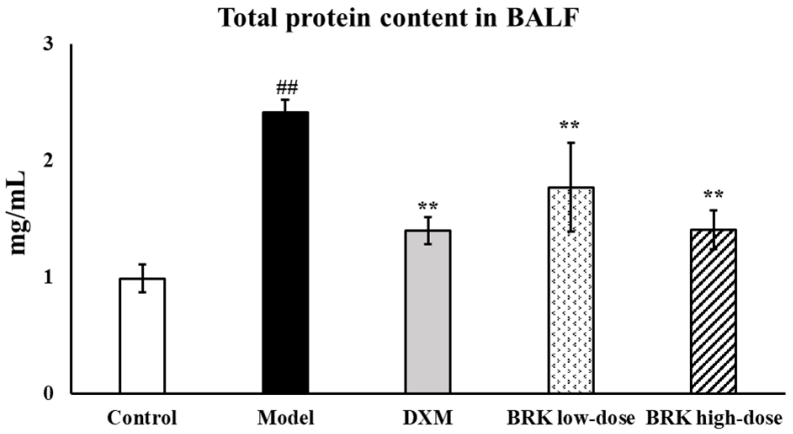

Figure 2 BRK treatment decreased the total protein content in BALF in Poly(I:C)-induced acute lung injury mice. Control, Model, DXM, BRK low-dose and BRK high-dose groups (n = 6 per group). ##: $P < 0.01$ compared with Control group; **: $P < 0.01$ compared with Model group.

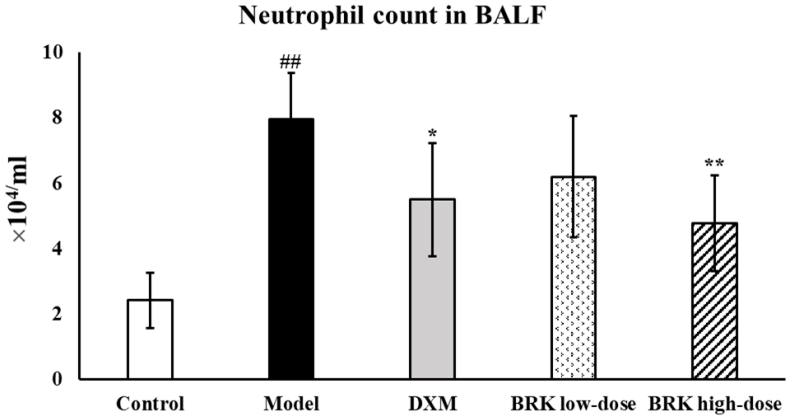

Figure 3 BRK treatment decreased the neutrophil count in BALF in Poly(I:C)-induced acute lung injury mice. Control, Model, DXM, BRK low-dose and BRK high-dose groups (n = 6 per group). ##: $P < 0.01$ compared with Control group; *: $P < 0.05$ compared with Model group; **: $P < 0.01$ compared with Model group.

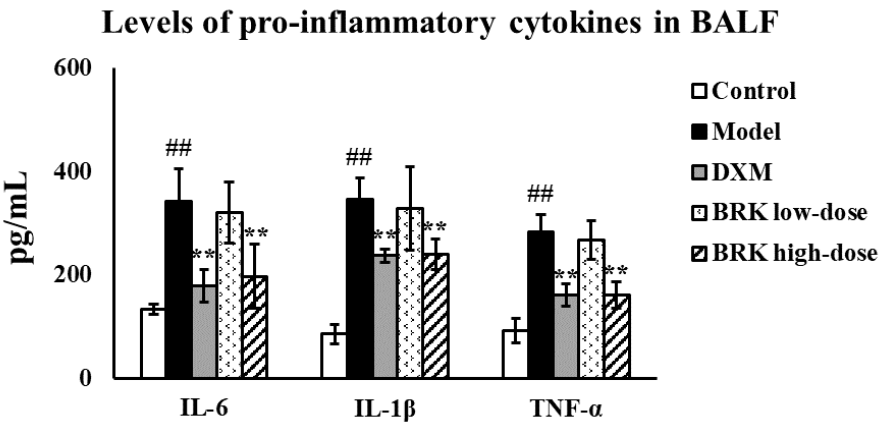

Figure 4 BRK treatment decreased the levels of pro-inflammatory cytokines in BALF in Poly(I:C)-induced acute lung injury mice. Control, Model, DXM, BRK low-dose and BRK high-dose groups (n = 6 per group). ##: $P < 0.01$ compared with Control group; **: $P < 0.01$ compared with Model group.

Discussion

ALI is a clinical syndrome caused by characteristic pathological changes in lung tissues due to severe infection, shock, and trauma. The clinical manifestations of ALI are diffuse lung exudation and refractory hypoxemia. Inflammation-induced tissue injury is the pathological basis of ALI, and the characteristic marker of a local inflammation is the aggregation of a considerable number of cells, and many cytokines play important roles in neutrophil aggregation. ALI is often accompanied by inflammation and the release of many inflammatory cytokines, resulting in neutrophil infiltration. The BALF neutrophil count can directly reflect the neutrophil exudation and aggregation in the lungs [5–7]. After the intratracheal injection of POLY(I:C), inflammation-related changes rapidly occurred in mouse lungs, which manifested as an increase in local IL-6, IL-1β, and TNF-α concentrations, interalveolar protein exudation, lung edema, and increase in lung W/D ratio. In addition, the lung structure underwent rapid destruction, where the local alveolar structure disappeared, the local alveolar septum was significantly thickened, and interstitial bleeding could be seen in mice after the intratracheal injection of LPS. The treatment using the BRK and dexamethasone alleviated the POLY(I:C)-induced lung injury, decreased the lung exudation and edema, and significantly decreased the BALF neutrophil count; IL-6, IL-1β, and TNF-α concentrations; and neutrophil infiltration in the lungs.

In this study, POLY(I:C) was used to construct an ALI mouse model. Our results showed that the BRK can inhibit neutrophil infiltration in the lungs, decrease IL-6, IL-1β, and TNF-α production, and prevent lung apoptosis in ALI mice.

In summary, the BRK could possibly treat ALI.

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