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Jolkinolide B attenuates allergic airway inflammation and airway remodeling in asthmatic mice

Haiyan Lin[a], Chao Xu[b], Jintong Ge[c], Hua Wu[c], Qi Wang[c]*

[a]*Department of Respiratory and Critical Care Medicine, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, Huai'an, Jiangsu, China*
[b]*Department of Pediatric Surgery, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, Huai'an, Jiangsu, China*
[c]*Department of Thoracic Surgery, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, Huai'an, Jiangsu, China*

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Abstract
Asthma is a widely prevalent chronic disease that brings great suffering to patients and may result in death if it turns severe. Jolkinolide B (JB) is one diterpenoid component separated from the dried roots of Euphorbia fischeriana Steud (Euphorbiaceae), and has anti-inflammatory, antioxidative, and antitumor properties. However, the detailed regulatory role and associated regulatory mechanism in the progression of asthma remain elusive. In this work, it was demonstrated that the extensive infiltration of bronchial inflammatory cells and the thickening of airway wall were observed in ovalbumin (OVA)-induced mice, but these impacts were reversed by JB (10 mg/kg) treatment, indicating that JB relieved the provocative symptoms in OVA-induced asthma mice. In addition, JB can control OVA-triggered lung function and pulmonary resistance. Moreover, JB attenuated OVA-evoked inflammation by lowering the levels of interleukin (IL)-4, IL-5, and IL-13. Besides, the activated nuclear factor kappa B (NF-κB) and transforming growth factor-beta-mothers against decapentaplegic homolog 3 (TGFß/smad3) pathways in OVA-induced mice are rescued by JB treatment. In conclusion, it was disclosed that JB reduced allergic airway inflammation and airway remodeling in asthmatic mice by modulating the NF-κB and TGFß/smad3 pathways. This work could offer new opinions on JB for lessening progression of asthma.

***Corresponding author:** Qi Wang, Department of Thoracic Surgery, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, No. 1, Huanghe West Road, Huai'an, Jiangsu 223000, China. *Email address*: hayywq@njmu.edu.cn

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Introduction

Asthma is a chronic and heterogeneous disease that influences humans at different ages and is featured by the presence of chronic inflammation, mucus hypersecretion, and airway hyperresponsiveness.[1,2] The disease is frequently accompanied by distressing symptoms (such as chest tightness, wheezing, difficulty in breathing, and cough) and is life-threatening in severe cases.[3] The prevalence of asthma has steadily exaggerated in recent years, and it is estimated that about 400 million people would be affected by this disease globally at 2025.[4] Inhaled corticosteroids is the main drug for asthma treatment, but these need frequent administration because of their short-acting properties.[5] Thus, there is an urgent need for novel therapeutic agents to effectively control symptoms and exacerbations in asthma patients.

Jolkinolide B (JB), a type of diterpenoid component isolated from the dried roots of Euphorbia Fischeriana Steud (Euphorbiaceae), has anti-inflammatory, antioxidative, and antitumor properties.[6] Researchers have discovered the regulatory effects of JB to mitigate the progression of multiple diseases. For instance, JB modulates the JAK2/STAT3 pathway to relieve the progression of rheumatoid arthritis.[7] Additionally, JB exhibits protective functions on lipopolysaccharide (LPS)-induced acute lung injury.[8] Furthermore, in mice with unilateral urethral obstruction, JB is discovered to restrain inflammation and epithelial-mesenchymal transition to weaken renal fibrosis.[9] JB retards the phosphatidylinositol 3-kinase–protein kinase B (PI3K-Akt) pathway to refrain cell proliferation and accelerate cell apoptosis in carcinoma of the breast.[10] Besides, JB affects the expression of Musashi-2 protein to retard the development of hepatocellular carcinoma.[11] Nevertheless, the detailed regulatory function and associated regulatory pathways of JB in checking the progression of asthma remained vague. Therefore, we investigated the regulatory functioning of JB in controlling the progression of asthma, and speculated that JB could mitigate asthma. In this regard, first it was revealed that JB attenuated allergic airway inflammation and airway remodeling in asthmatic mice by modulating the nuclear factor kappa B (NF-κB) and transforming growth factor-beta–mothers against decapentaplegic homolog 3 (TGFß/smad3) pathways. This work suggested that JB could be helpful for the treatment of asthma.

Materials and methods

Asthma mouse model

In all, 24 BALB/c mice (6-week-old males) were used in the study (Vital River Laboratory Animal Technology Co. Ltd., Beijing, China). Mice were fed with free diet and water under 12-h light–dark cycle and 23 ± 3°C. All procedures were approved by the Animal Welfare Ethics Committee of the Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, China. The mice were randomly divided into the following four groups (n = 6 mice in each group): control, OVA, OVA+JB (2.5 mg/kg), and OVA+JB (10 mg/kg).

The ovalbumin (OVA, grade V; Sigma, MA, USA) was utilized to stimulate asthma mouse model. On days 0 and 7, OVA (100 μg/2 mg Imject™ alum adjuvant) diluted in phosphate-buffered saline solution (PBS, 200 μL) was intraperitoneally injected into mice. On days 14, 17, and 20, OVA (100 μg) in PBS (20 μL) was intratracheally sued in mice; then, twice a week for 4 weeks. In the control group, at the same time points, mice were sensitized and challenged by using PBS. JB ($C_{28}H_{26}O_4$; 2.5 or 10 mg/kg, purity > 99%; Nanjing Daosifu Biotechnology Co. Ltd, Nanjing, China) dissolved in normal saline (200 μL) was administered orally to mice every day from day 15–49. Finally, the lung function parameters were discovered, and all mice were sacrificed.

Hematoxylin and eosin (H&E) staining

The bronchovesicular tissues of mice were dehydrated, permeabilized, embedded, and sliced. The 4-μm sections were dyed with hematoxylin (5 min) and then dyed with eosin (2 min). Finally, the changes in bronchovesicular tissues were inspected under light microscope (Olympus Corporation, Tokyo, Japan).

Detection of lung functions and airway resistance (AWR)

After being anesthetized with sodium pentobarbital (50 mg/kg), the tracheal intubation was performed using 22-G indwelling needle. Next, the mouse lung function instrument was connected, and the assisted respiration was induced through a respirator. The lung function indicators, such as peak expiratory flow (PEF), forced expiratory volume in 0.4 s (FEV 0.4) to forced vital capacity (FVC; [FEV0.4/FVC]), and respiratory rate, were measured. Methacholine (3.12, 6.25, 12.5, and 25 mg/mL) was injected intravenously (i.v.) to evaluate AWR (cm H_2O/mL/s).

Cell counting

Lavage fluid (10 μL) was centrifugated, and cell sediment was resuspended by PBS to obtain cell suspension (100 μL). After removing the liquid, cells were dried and dyed with Diff-Quik solution. The number of cells (total cells and eosinophils) was counted under cell counter.

Enzyme-linked-immunosorbent serologic assay (ELISA)

The ELISA kits of interleukin (IL)-4 (ab100710; Abcam, Shanghai, China), IL-5 (ab204523), and IL-13 (ab219634) were applied to assess the levels of IL-4, IL-5, and IL-13 in bronchoalveolar lavage fluid (BALF).

Masson staining

The 4-μm sections of paraffin-embed bronchovesicular tissues were dyed with Masson's trichrome staining solution

(Sigma, St Louis, MO, USA) following the manufacturer's instructions.

Western blot analysis

Proteins from bronchovesicular tissues were extracted by radioimmunoprecipitation assay (RIPA) lysis buffer. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to separate proteins; then, the proteins were transferred to polyvinylidene difluoride membrane (PVDF; Millipore, Billerica, MA, USA). The membranes were incubated with primary antibodies for 12 h, then with a secondary antibody (1/1000; ab6721) for 2 h. Finally, the enhanced chemiluminescence kit (Thermo Fisher Scientific, MA, USA) was used to visualize the bands, which were then quantified by Image J (National Institutes of Health, Bethesda, MD, USA).

The primary antibodies were as follows: α-SMA (1/10,000; ab124964; Abcam), phosphorylated (p)-p65 (RelA, 1/2000; ab76302; Abcam), p65 (0.5 μg/mL; ab16502; Abcam), TGFß1 (1/1000; ab215715; Abcam), phosphorylated (p)-smad3 (1/1000; ab63402; Abcam), smad3 (1 μg/mL; ab84177; Abcam), and ß-actin (1 μg/mL; ab8224; Abcam).

Statistical analysis

The data were expressed as mean ± standard deviation (SD). The GraphPad Prism Software 9 (GraphPad Software, USA) was utilized for statistical analysis. The one-way analysis of variance (ANOVA) was adopted for analyzing differences in groups; $P < 0.05$ was considered statistically significant.

Results

JB alleviated provocative symptoms in OVA-induced asthma mice

Through H&E staining, the extensive infiltration of bronchial inflammatory cells and thickening of the airway wall were observed in OVA-induced mice, but these impacts were reversed by JB (10 mg/kg) treatment (Figure 1). Thus, JB relieved provocative symptoms in OVA-induced asthma mice.

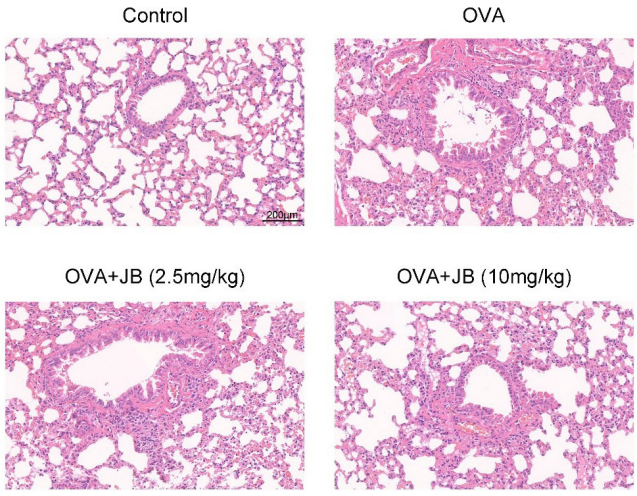

Figure 1 JB alleviated provocative symptoms in OVA-stimulated asthma mice. Mice were divided into control, OVA, OVA+JB (2.5 mg/kg), and OVA+JB (10 mg/kg) groups. Pathological changes in pulmonary bronchus were examined by Hematoxylin and eosin (H&E) staining.

JB controlled OVA-triggered lung function and pulmonary resistance

The PEF and $FEV_{0.4}$/FVC measures were decreased and the respiratory rate was elevated in OVA-induced mice, but these changes were neutralized by JB (10 mg/kg) treatment (Figure 2A). Furthermore, the AWR was increased in OVA-induced mice, but this effect was weakened by JB (10 mg/kg) treatment (Figure 2B). Taken together, JB controlled OVA-triggered lung function and pulmonary resistance.

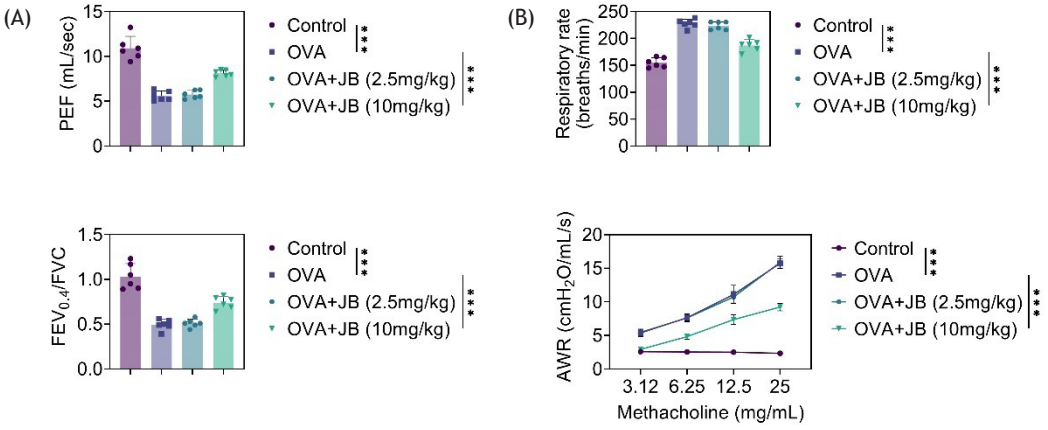

Figure 2 JB improved OVA-triggered injury in lung function and pulmonary resistance. Mice were divided into control, OVA, OVA+JB (2.5 mg/kg), and OVA+JB (10 mg/kg) groups. (A) Lung functions (PEF, $FEV_{0.4}$/FVC, and respiratory rate) were confirmed. (B) Airway resistance (AWR) was inspected by using different concentrations of methacholine (3.12, 6.25, 12.5, and 25 mg/mL). $^{***}P < 0.001$.

JB attenuated OVA-evoked inflammation

Total cells and eosinophils were exaggerated after OVA induction, but these changes were neutralized by JB (10 mg/kg) treatment (Figure 3A). Moreover, the levels of IL-4, IL-5, and IL-13 were augmented by OVA treatment, but these effects were relieved by JB (10 mg/kg) treatment (Figure 3B). In general, JB attenuated OVA-induced inflammation.

JB relieved OVA-induced airway remodeling

Through Masson staining, the accumulation of collagen fibers in pulmonary bronchus was increased in OVA-induced mice, but this phenomenon was mitigated by JB (10 mg/kg) treatment (Figure 4A). In addition, the smooth muscle alpha-actin (α-SMA) protein expression was raised by OVA stimulation, which was relieved by JB (10 mg/kg) treatment (Figure 4B). To sum up, JB relieved OVA-induced airway remodeling.

JB retarded NF-κB and TGFβ/smad3 pathways

The p-p65/p65 level was strengthened by OVA stimulation, which was reversed by JB (10 mg/kg) treatment (Figure 5A). Besides, the protein expressions of both TGFβ1 and p-smad3/smad3 pathways were intensified, but these effects were weakened by JB (10 mg/kg) treatment (Figure 5B). In short, JB retarded NF-κB and TGFβ/smad3 pathways.

Discussion

Jolkinolide B has been testified to exhibit anti-inflammatory, antioxidative, and antitumor functions in diversiform diseases.[7-11] However, the detailed regulatory roles and associated regulatory pathways of JB in controlling the progression of asthma remained indistinct. Hence, we investigated the regulatory effect of JB on asthma progression, and speculated that JB could mitigate asthma. In the present study, it was demonstrated that the extensive infiltration of bronchial inflammatory cells and thickening of the airway wall were observed in OVA-induced asthmatic mice, but these impacts were reversed by JB (10 mg/kg) treatment, indicating that JB alleviated agitative symptoms in OVA-induced asthmatic mice. In addition, JB controlled OVA-triggered lung function and pulmonary resistance.

Inflammation is a pivotal process in the progression of asthma.[12] Researchers have paid more attention on the regulation of inflammation to control asthma. For example, formononetin (an O-methylated isoflavone) checks murine allergic asthma by alleviating airway inflammation and oxidative stress.[11] In addition, oroxylin A (an O-methylated flavone) restrains allergic airway inflammation to lessen OVA-stimulated asthma.[13] Ligustrazine (tetramethylpyrazine) mitigates inflammation in OVA-mediated asthma.[14] Moreover, osthole (derivative of coumarin) suppresses the IL-33/ST2 signaling to alleviate OVA-induced lung inflammation in asthmatic mice.[15] Similar to previous studies, in the present work, it was demonstrated that JB attenuated OVA-evoked inflammation by decreasing IL-4, IL-5, and IL-13 levels.

NF-κB is a central mediator of inflammatory response.[16] Under normal circumstances, NF-κB is restrained by nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) at resting state; However, activation of inflammation results in IκBα degradation, thereby activating NF-κB.[17] The long-term activation of NF-κB has been implicated in the development of asthma.[18]

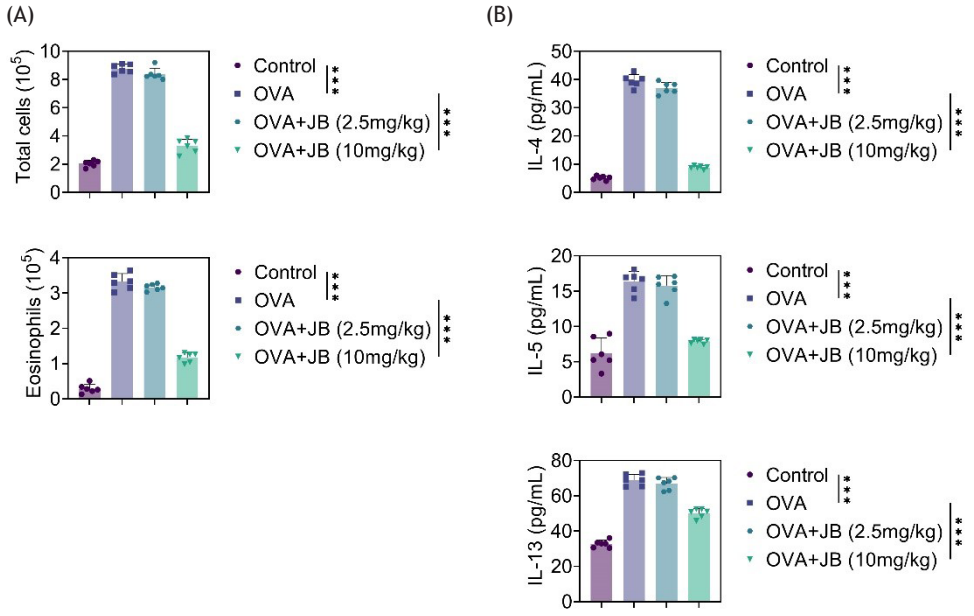

Figure 3 JB attenuated OVA-evoked inflammation. Mice were divided into Control, OVA, OVA+JB (2.5 mg/kg), and OVA+JB (10 mg/kg) groups. (A) Total cells and eosinophils were measured through cell counter. (B) Levels of IL-4, IL-5, and IL-13 in the supernatant of bronchoalveolar lavage fluid (BALF) were tested through ELISA. ***P < 0.001.

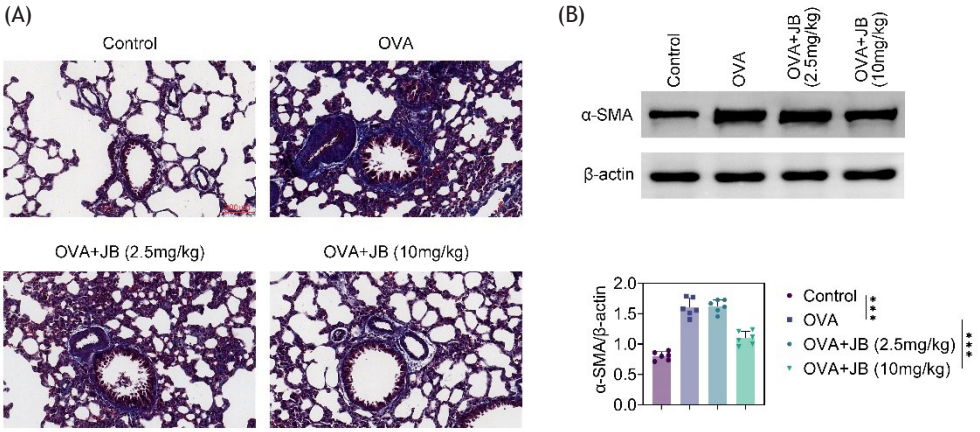

Figure 4 JB relieved OVA-induced airway remodeling. Mice were divided into control, OVA, OVA+JB (2.5 mg/kg), and OVA+JB (10 mg/kg) groups. (A) Pathological changes in pulmonary bronchus were observed through Masson staining. (B) The protein expression of α-SMA was determined through Western blot analysis. ***$P < 0.001$.

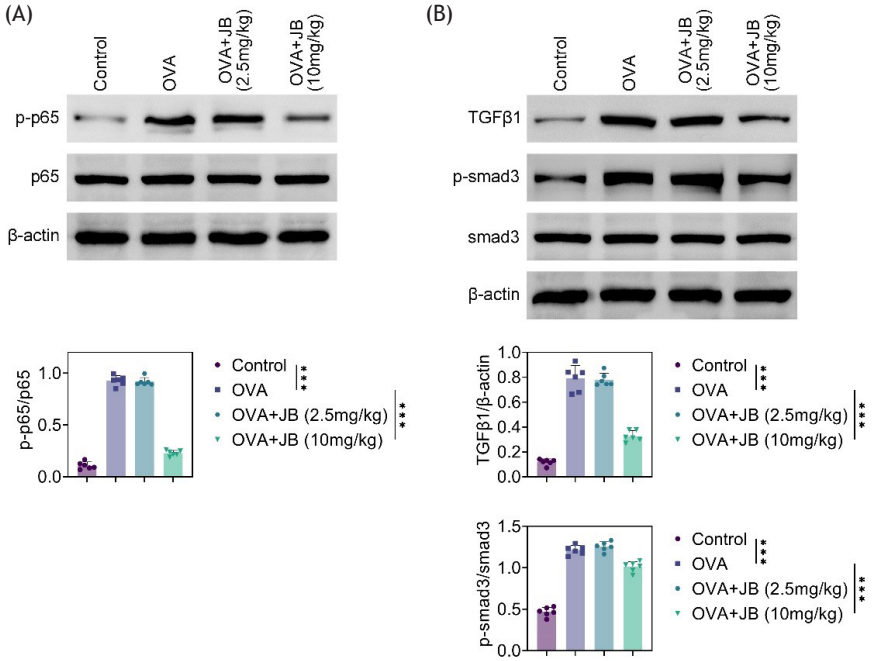

Figure 5 JB retarded NF-κB and TGFβ/smad3 pathways. Mice were divided into control, OVA, OVA+JB (2.5 mg/kg), and OVA+JB (10 mg/kg) groups. (A) The protein expressions of p-p65 and p65 were examined through Western blot analysis. (B) The protein expressions of TGFβ1, p-smad3, and smad3 were also assessed through Western blot analysis. ***$P < 0.001$.

TGFβ1 is a profibrotic cytokine that takes part in the pathogenesis of asthma.[19] It has been reported that TGFβ1-positive cells exist in the bronchial biopsies of severe asthma, and retarding of TGFβ1 signaling is an important mechanism to inhibit asthmatic airway remodeling.[20] The TGFβ/smad3 pathway is determined to take part in the regulation of asthma. For instance, Fangxiao Formula (FXF) retards the TGFβ/smad3 pathway to relieve airway inflammation and remodeling in asthma.[21] Moreover, in asthma, riparin II affects the TGFβ/smad3 pathway to influence ephedrine's functioning on inflammation and remodeling.[22] Besides, syndecan-1 fortifies the action of TGFβ1/smad3 pathway in asthma to heighten OVA-triggered airway remodeling.[23] In the present study, it was discovered that the activated NF-κB and TGFβ/smad3 pathways in OVA-induced asthmatic mice could be relieved by JB treatment.

Conclusion

It was uncovered that JB attenuated allergic airway inflammation and airway remodeling in asthmatic mice by modulating the NF-κB and TGFβ/smad3 pathways. Nevertheless, this work has some limitations, such as lacking clinical investigations, cell modeling, human samples, and other phenotypic investigations. More advanced investigations concerning functioning of JB in asthma progression are required in the future.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request. Data sharing is not applicable as no new data were created or analyzed in this study.

Competing interests

The authors stated that there was no conflict of interest to disclose.

Author Contributions

All authors contributed to the study's conception and design. Material preparation and the experiments were conducted by Haiyan Lin. Data collection and data analysis were performed by Chao Xu, Jintong Ge, and Hua Wu. The first draft of the manuscript was prepared by Qi Wang. All authors commented on previous versions of the manuscript, and read and approved the final manuscript.

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