



Berberine improves airway inflammation and inhibits NF- κ B signaling pathway in an ovalbumin-induced rat model of asthma

Zhenghao Li MMed, Jie Zheng MB, Ning Zhang MMed & Chengde Li MMed

To cite this article: Zhenghao Li MMed, Jie Zheng MB, Ning Zhang MMed & Chengde Li MMed (2016) Berberine improves airway inflammation and inhibits NF- κ B signaling pathway in an ovalbumin-induced rat model of asthma, Journal of Asthma, 53:10, 999-1005, DOI: [10.1080/02770903.2016.1180530](https://doi.org/10.1080/02770903.2016.1180530)

To link to this article: <http://dx.doi.org/10.1080/02770903.2016.1180530>



Accepted author version posted online: 13 May 2016.
Published online: 13 May 2016.



Submit your article to this journal [↗](#)



Article views: 61



View related articles [↗](#)



View Crossmark data [↗](#)

ALTERNATIVE MEDICINE

Berberine improves airway inflammation and inhibits NF- κ B signaling pathway in an ovalbumin-induced rat model of asthma

Zhenghao Li, MMed¹, Jie Zheng, MB¹, Ning Zhang, MMed¹, and Chengde Li, MMed²

¹Department of Neonatology, Yidu Central Hospital, Qingzhou, Shandong, China, and ²Department of Pharmacology, Key Laboratory of Applied Pharmacology of Shandong Province, Weifang Medical University, Weifang, Shandong, China

Abstract

Objectives: Berberine has been reported for its various activities including anti-inflammatory effects and has been used in treating many diseases. However, its effects on airway inflammation in asthma have not been investigated. This study mainly aimed to detect its effects on the airway inflammation and the nuclear factor- κ B (NF- κ B) signaling pathway activity in a rat model of asthma. **Methods:** Asthma was induced by ovalbumin (OVA) sensitization and challenge. The asthmatic rats were respectively treated with vehicle PBS or berberine (100 mg/kg or 200 mg/kg) for 28 days. The control rats were treated with PBS. Inflammatory cells in bronchoalveolar lavage fluid (BALF) were counted and the lung inflammation was scored. Levels of NF- κ B p65 (mRNA and protein), phosphorylated NF- κ B p65 (p-NF- κ B p65), inhibitory κ B α (I κ B α) (mRNA and protein) and phosphorylated I κ B α (p-I κ B α), as well as NF- κ B p65 DNA-binding activity, were measured to assess the activity of NF- κ B signaling pathway. Levels of the downstream inflammatory mediators of NF- κ B signaling, IL-1 β , IL-4, IL-5, IL-6, IL-13 and IL-17 in BALF, were measured. Besides, the serum levels of OVA-specific immunoglobulin (Ig)E were measured. **Results:** Results showed that OVA increased the number of inflammatory cells in BALF, elevated lung inflammation scores, enhanced the NF- κ B signaling activity and promoted the production of IgE in rats. Berberine dose-dependently reversed the alterations induced by OVA in the asthmatic rats. **Conclusions:** The findings suggested a therapeutic potential of berberine on OVA-induced airway inflammation. The ameliorative effects on the OVA-induced airway inflammation might be associated with the inhibition of the NF- κ B signaling pathway.

Introduction

Allergic asthma is a chronic pulmonary inflammatory disease characterized by lung infiltration of macrophages, eosinophils, lymphocytes and neutrophils [1]. Glucocorticoids are the major therapy for asthma, but they have many adverse reactions. In addition, glucocorticoids are not effective for some patients, especially the patients with severe asthma (5%–10% of asthmatics) [2]. For these asthmatic patients, there is a need for alternative or adjuvant drugs targeting the airway inflammation.

Complex interactions of immune cells have important roles in the development and progression of airway inflammation. These cells can augment the inflammatory response by producing a number of inflammatory mediators, such as Th2-associated cytokines [3], Th17-associated cytokines [4], and some other pro-inflammatory cytokines [5]. Actually, in addition to macrophages, eosinophils, mast cells, Th2 cells and Th17 cells, the structural cells in the airways are also important sources of cytokines. Besides promoting inflammatory

Keywords

Berberine, effect, airway inflammation, asthma, NF- κ B signaling pathway

History

Received 24 January 2016

Revised 11 April 2016

Accepted 16 April 2016

cascade reactions, these cytokines also contribute to many other pathophysiological features of asthma, such as goblet cell mucus secretion and airway remodeling. Modulating the cytokine network has been proven to present a new but challenging paradigm for treatment of asthma [6].

The cytokine network can be activated by NF- κ B, a potent inflammatory transcription factor that regulates a wide variety of target genes. NF- κ B consists of homo- or heterodimers of NF- κ B1/p50, NF- κ B2/p52, RelA/p65, Rel/c-Rel, and RelB [7]. Among the NF- κ B components, NF- κ B p65 is a key active subunit. In the NF- κ B signal transduction pathway, I κ B α is a negative regulator of NF- κ B. NF- κ B is activated when I κ B α is phosphorylated by I κ B kinase. The activated NF- κ B translocates into the nucleus where it promotes gene transcription, leading to the overproduction of some inflammatory cytokines. It has been reported that the expression of IL-1, IL-6, IL-4, IL-5, IL-13 and IL-17 are all associated with the activation of the NF- κ B signaling [8–11]. In turn, these inflammatory cytokines can also promote the activation of NF- κ B signaling, which results in complex inflammatory cascade reactions in the body [12,13].

It has been well demonstrated that the asthmatic individuals have increased levels of NF- κ B activity in allergen-challenged lungs, airway epithelial cells and macrophages [14]. Furthermore, there is evidence that NF- κ B activation is linked to

Correspondence: Zhenghao Li, Department of Neonatology, Yidu Central Hospital, Qingzhou, Shandong Province 262500, China. E-mail: sdqdcqq@126.com

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ijas.

uncontrolled/acute exacerbations of asthma [15]. Based on the crucial role of NF- κ B signaling in asthma, suppressing the over-activation of the NF- κ B signaling pathway has been tested as new therapeutic targets for asthma [16,17].

Many natural compounds that possess anti-inflammatory activities, such as puerarin, matrine, astragaloside, resveratrol and curcumin, have been tested to treat asthma [18,19]. These compounds can ameliorate asthma by various mechanisms. Berberine is an extract from herbal medicine *Coptis chinensis*. Berberine has exhibited anti-oxidative, anti-inflammatory, renoprotective, neuroprotective and some other activities in animal models and cellular systems. It is now used to manage many diseases in clinic [20,21].

In an *in vitro* study, Lee CH found that berberine could suppress the productions of pro-inflammatory cytokines induced by okadaic acid, lipopolysaccharide, hydrogen peroxide, or ceramide via the inhibition of I κ B degradation in human lung cells [22]. Two recent animal studies showed that berberine attenuated cigarette smoke-induced lung inflammation in mice [23,24]. However, it has not been investigated whether berberine can attenuate the airway inflammation in subjects with asthma and whether this effect is associated with the inhibition of NF- κ B signaling pathway.

Thus, this study was conducted to investigate the possible effects of berberine on airway inflammation and the activity of NF- κ B signaling pathway in an OVA- induced rat model of asthma.

Methods

Animals

Forty male Wistar rats (Laboratory Animal Center of Lukang Company, Jining, China) weighing 220–260 grams were used in this study. Rats were housed in temperature- controlled room ($24 \pm 2^\circ\text{C}$) with *ad libitum* access to food and water on a 12:12 light-dark cycle. The protocols of the studies conformed to the requirements of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of Yidu Central Hospital. The animals were randomly divided into four groups ($n = 10$ per group): control group, vehicle group, berberine low-dose group (low-dose group) and berberine high-dose group (high-dose group).

Induction of asthma by OVA sensitization and challenge

The induction of asthma in rats was conducted as previously described [25]. Briefly, rats in the vehicle group, the low-dose group and the high-dose group were sensitized on day 1 and day 8 by intraperitoneal injection of 1 mg OVA (Sigma Chemical Co., St. Louis, MO, USA) and 10 mg of aluminium hydroxide (Thermo Scientific Pierce, Rockford, Rockford, IL, USA) suspended in 2 ml phosphate-buffered saline (PBS.) Seven days after the last OVA immunization, rats were challenged with 1% OVA aerosol (dissolved in PBS) generated using an ultrasonic nebulizer for 28 days (20 minutes per day). Rats in the control group were sensitized and challenged with PBS only. Figure 1 is a flow diagram of the protocol.

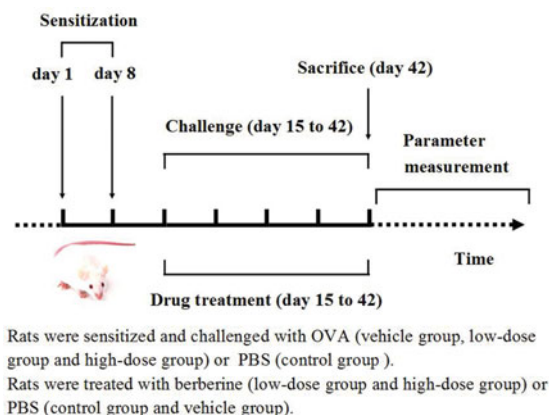


Figure 1. The flow diagram of the protocol of the study.

Drug treatment

Berberine solution dissolved in PBS was daily given (orally) to the rats in the low-dose group (100 mg/kg body weight) and the high-dose group (200 mg/kg body weight) 2 hours before each 1% OVA aerosol challenge for 28 successive days. Rats in the control group and the vehicle group were treated with the same volume of PBS only. Figure 2 shows the chemical structure of berberine.

Inflammatory cell counts in BALF

The animals were sacrificed via overdose of pentobarbital. The left lung was removed and BALF was prepared by washing lungs with ice-cold PBS as described elsewhere [26]. The BALF was immediately centrifuged at 3000 rpm for 10 min at 4°C . The supernatant was collected and stored at minus 80°C for cytokine investigations. Cells in the BALF were stained with Wright–Giemsa staining. Differential cell count was performed by two investigators who were blind to the groups.

Lung tissue histology

The lower lobe of the right lung was ligatured and removed for NF- κ B signaling assessment. The other part of the right lung was inflated with 4% paraformaldehyde at the pressure of 20 cm H_2O and fixed in the 4% paraformaldehyde solution for 24 h. Then the paraformaldehyde-fixed lung tissue was embedded in paraffin. The middle lobe of the right lung was

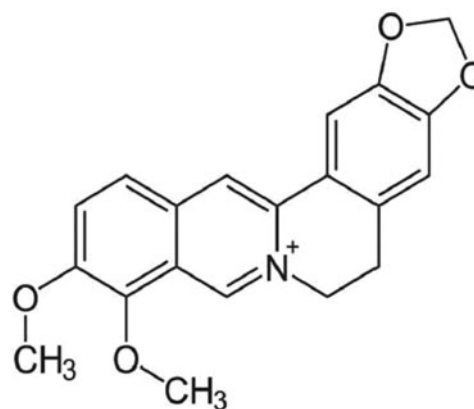


Figure 2. Chemical structure of berberine.

used for the histological analysis. Serial 5- μ m sections were stained with hematoxylin and eosin. The inflammation was scored on the scales 0–3 based on the distribution and severity of inflammation as described elsewhere [26,27]: Scale 0: no inflammation was detectable; scale 1: occasionally inflammatory cells was detectable; scale 2: most bronchi were surrounded by a thin layer (1–5 cells) of inflammatory cells; scale 3: most bronchi or vessels were surrounded by a thick layer (more than 5 cells) of inflammatory cells. Five randomly selected tissue sections were analyzed for each rat by two investigators who were blind to the groups under the light microscopy.

Assessment of the activity of NF- κ B signaling pathway

Tissue samples from the right lung were homogenized (100 mg tissue/1 ml saline) and centrifuged. The supernatant was collected. Commercially available ELISA kits (Bangyi Biotechnology Co. Ltd., Shanghai, China; Cell Signaling Technology, Beverly Massachusetts, USA) were used to measure the levels of NF- κ B p65, p-NF- κ B p65, I κ B α and p-I κ B α as described in literature [28,29].

Detection of NF- κ B p65 and I κ B α mRNA expression in lung

Total tissue RNA was extracted from the right lung tissue samples with TRIzol reagent (TaKaRa Bio, Dalian, China). The cDNA synthesized from 2 μ g total RNA was amplified through quantitative real-time reverse transcription PCR analysis. The NF- κ B p65 primer sequences were: forward 5'-TGCAGGCTCCTGTGCGAGTG-3' and NF- κ B p65 reverse 5'-TCCGGTGGCGATCGTCTGTGT-3'. The I κ B α primer sequences were: forward 5'-CGTGTCTGCACCTAGCCTCTATC-3' and I κ B α reverse 5'-GCGAAACCAGGTCAGGATTC-3'. The β -actin primer sequences were: forward 5'-GCAGGAGTACGATGAGTCCG-3' and reverse 5'-ACGCAGCTCAGTAACAGTCC-3'. The PCR program involved 95°C for 30 s and 40 PCR cycles (95°C for 5 s and 60°C for 30 s). The NF- κ B p65 mRNA expression was normalized to β -actin.

NF- κ B p65 DNA binding activity

Nuclear protein was extracted from fresh lung tissue using a nuclear extraction kit (Active Motif, Carlsbad, CA, USA). Nuclear protein (10 μ g) was used to detect NF- κ B p65 DNA-binding activity using TranAMTM NF- κ B p65 Chemi transcription factor assay kits (Active Motif, Carlsbad, CA, USA). The NF- κ B p65 DNA-binding activity was determined by measuring the absorbance at 450.

Measurement of cytokine levels in BALF

Concentrations of cytokines IL-1 β , IL-4, IL-5, IL-6, IL-13 and IL-17 in BALF were measured using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions (Nanjing Jiancheng Company, Nanjing, China; R&D Systems, Inc., Minneapolis, MN, USA).

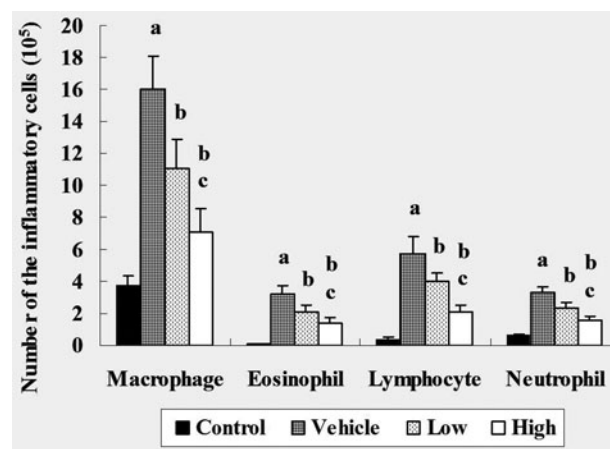


Figure 3. Changes of the number of inflammatory cells in BALF. Data are expressed as means $\pm$ SD. aP < 0.05 versus control group; bP < 0.05 versus vehicle group; cP < 0.05 versus low dose group.

Detection of OVA-specific IgE levels in serum

Blood was collected by cardiac puncture and centrifuged to prepare serum. OVA-specific IgE levels in serum were measured using ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA).

Statistical analysis

Data were expressed as mean $\pm$ SD and analyzed using SPSS software 13.0. Statistical comparisons were made using a one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls (SNK) test. P < 0.05 was considered statistically significant.

Results

Berberine reduced the number of inflammatory cells in BALF

As shown in Figure 3, the vehicle group had much more eosinophils, macrophages, neutrophil and lymphocytes in BALF if compared to the control group (P < 0.05). Berberine significantly decreased the number of all these inflammatory cells in a dose-dependent manner in the low-dose group and the high-dose group, if compared to the vehicle group (P < 0.05).

Berberine attenuated the leukocyte infiltration in the lung tissue

The histological detection showed that the vehicle group had higher inflammation scores than the control group (P < 0.05), which meant there was significant infiltration of inflammatory cells in lung tissue of the asthmatic animals. Berberine significantly decreased the inflammation scores of the low-dose group and the high-dose group in a dose-dependent manner if compared to the vehicle group (P < 0.05), indicating that berberine could dose-dependently reduce the infiltration of inflammatory cells in the lung tissue of the asthmatic animals (Figure 4).

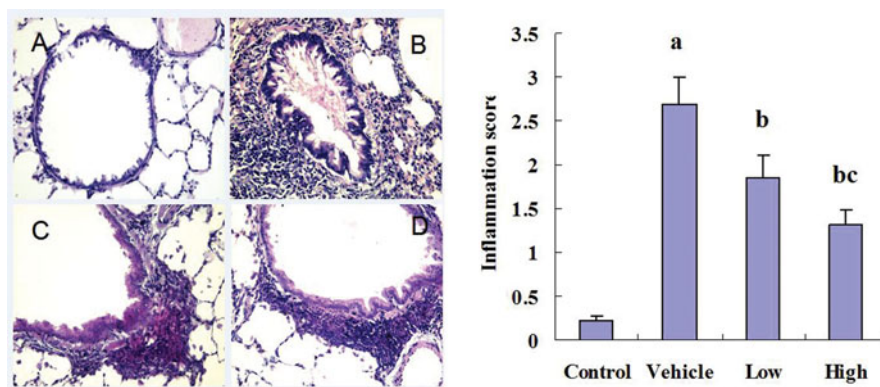


Figure 4. Inflammatory score of lung tissue (Hematoxylin and eosin staining $\times 200$). Data are expressed as means $\pm$ SD. aP < 0.05 versus control group; bP < 0.05 versus vehicle group; cP < 0.05 versus low dose group. A: control group; B: vehicle group; C: low dose group; D: high dose group.

Berberine inhibited the NF- κ B signaling

The expression of NF- κ B p65 (mRNA and protein), p-NF- κ B p65 and p-I κ B α were markedly increased in the vehicle group than the control group ($P < 0.05$). Berberine decreased their expression in a dose-dependent manner if compared to the vehicle group. But all the groups had similar I κ B α expression (mRNA and protein) ($P > 0.05$). In addition, the vehicle group exhibited elevated NF- κ B p65 DNA binding activity if compared to the control group ($P < 0.05$); whereas berberine decreased the NF- κ B p65 DNA binding activity in a dose-dependent manner if compared to the vehicle group ($P < 0.05$) (Figures 5, 6, and 7).

Berberine decreased the levels of pro- and inflammatory cytokines in BALF

Cytokines are the driving force behind cellular infiltration, airway remodeling, and airway hyperresponsiveness in asthma. Levels of IL-1 β , IL-4, IL-5, IL-6, IL-13 and IL-17 in BALF significantly increased in the vehicle group if compared to the control group ($P < 0.05$); whereas berberine decreased levels of these cytokines in a dose-dependent manner if compared to the vehicle group ($P < 0.05$) (Figure 8).

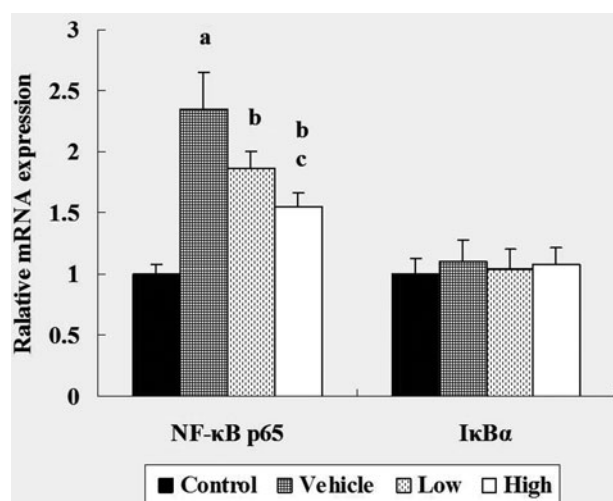


Figure 5. Levels of the mRNA expression of NF- κ B p65 and I κ B α of the rats. Data are expressed as means $\pm$ SD. aP < 0.05 versus control group; bP < 0.05 versus vehicle group; cP < 0.05 versus low dose group.

Berberine decreased the levels of OVA specific IgE

Animals in the vehicle group produced much higher levels of OVA-specific IgE than the control rats ($P < 0.05$). Berberine significantly inhibited the production of OVA-specific IgE

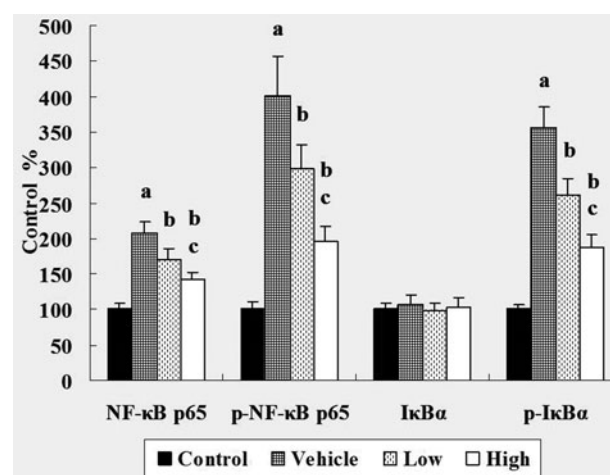


Figure 6. Levels of NF- κ B p65, p-NF- κ B p65, I κ B α and p-I κ B α of the rats. Data are expressed as means $\pm$ SD. aP < 0.05 versus control group; bP < 0.05 versus vehicle group; cP < 0.05 versus low dose group.

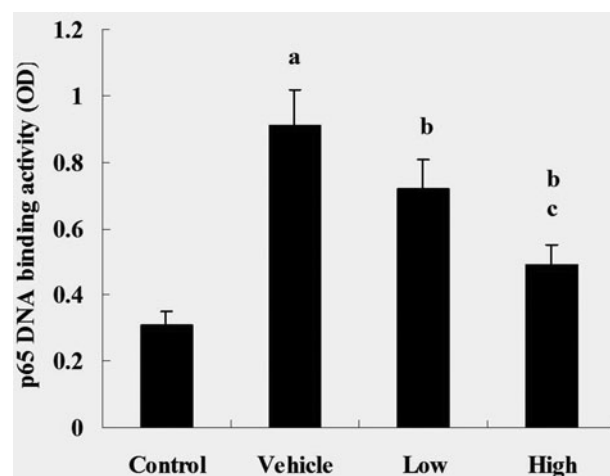


Figure 7. The NF- κ B p65 DNA binding activity of the rats. Data are expressed as means $\pm$ SD. aP < 0.05 versus control group; bP < 0.05 versus vehicle group; cP < 0.05 versus low dose group.

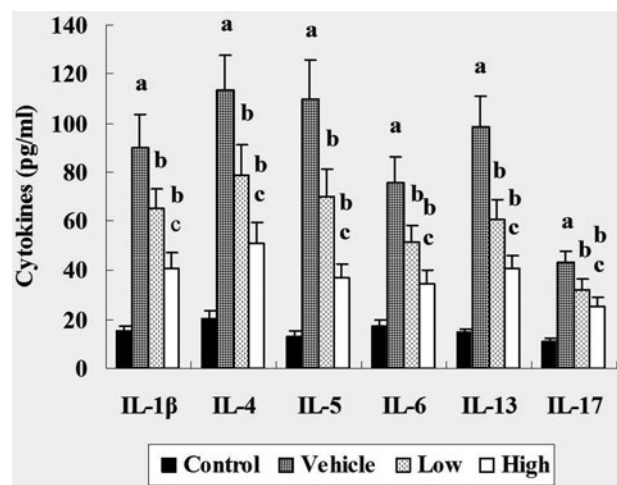


Figure 8. Levels of cytokines in BALF. Data are expressed as means $\pm$ SD. aP<0.05 versus control group; bP < 0.05 versus vehicle group; cP < 0.05 versus low dose group.

in the low-dose group and the high-dose group in a dose-dependent manner if compared to the vehicle group ($P < 0.05$) (Figure 9).

Discussion

Asthma is a chronic inflammatory airway disease with increasing prevalence [30]. Anti-inflammatory therapy is as one of the fundamental treatments for asthma [31]. The anti-inflammatory activity of berberine has been reported [22,32]. In this study, we demonstrate that berberine treatment attenuated the OVA-induced infiltration of eosinophils, macrophages, neutrophil and lymphocytes to the airway, inhibited the over-activation of NF- κ B signaling, and reduced the productions of cytokines IL-1 β , IL-6, IL-4, IL-5, IL-13 and IL-17 in Wistar rats.

NF- κ B signaling is a master regulator of inflammation and immune processes [33]. The activation of NF- κ B occurs when its negative regulator I κ B is phosphorylated by I κ B kinase. Dissociating from I κ B, the activated NF- κ B (p-NF- κ B) translocates into the nucleus and stimulates the transcription of its targeted genes [34]. There is increasing evidence that

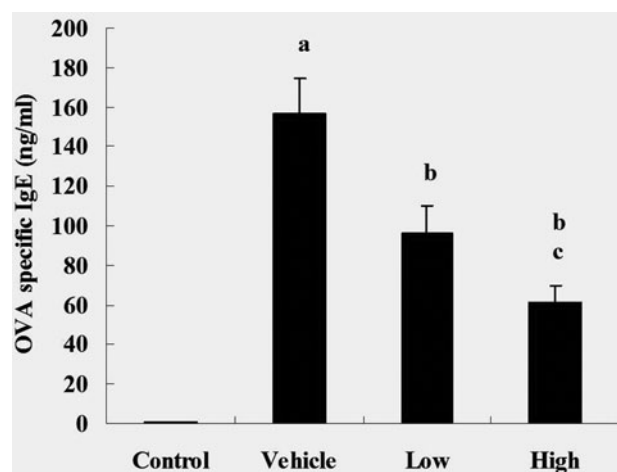


Figure 9. Levels of OVA-specific IgE in serum. Data are expressed as means $\pm$ SD. aP<0.05 versus control group; bP < 0.05 versus vehicle group; cP < 0.05 versus low dose group.

NF- κ B signaling is involved in asthma. Increased I κ B kinase expression, I κ B degradation (I κ B phosphorylation), NF- κ B nuclear localization and NF- κ B DNA binding activity have been found in the airway tissue of the asthmatics [35]. The OVA (an allergen which is widely used to induce asthma in animals) challenged animals also showed increased NF- κ B activation in the airway tissue and inflammatory cells [15,36,37].

Mice that lack the NF- κ B subunits developed less airway inflammation [8]. Moreover, suppression of the NF- κ B over-activation by specific NF- κ B inhibitor or other agents attenuated the lung inflammation in asthmatic animals [17,37,38]. All the evidence supports that NF- κ B signaling plays a crucial important role in asthma. In the current study, we found OVA sensitization and challenge significantly increased levels of NF- κ B p65, p-NF- κ B p65 and p-I κ B α in rats. Consistently, the NF- κ B p65 DNA binding activity also dramatically increased in the OVA-treated rats. The results were consistent with studies that reported the over-activation of the NF- κ B signaling in asthmatic animals and patients [15,36,37].

But, our results revealed that berberine treatment significantly decreased levels of NF- κ B p65, p-NF- κ B p65, p-I κ B α and NF- κ B p65 DNA binding activity in the asthmatic rats in a dose-dependent manner, suggesting that berberine could inhibit the NF- κ B signaling by decreasing the NF- κ B p65 expression and the I κ B α degradation (phosphorylation). In addition to the effects of berberine on the NF- κ B signaling we observed in the asthmatic rats, Liu W also found berberine decreased the NF- κ B expression and restored the I κ B α expression in alloxan-induced diabetic mice with renal injury [39]. Fu K found berberine hydrochloride attenuated lipopolysaccharide-induced endometritis in mice by suppressing activation of NF- κ B signaling pathway [40].

Over-production of some types of cytokines is one of the basic characteristics of asthma. The excessive cytokines induce a cascade of reactions in the airway and exacerbate asthma by promoting airway inflammation, inducing airway remodeling and increasing hyperresponsiveness. The roles of Th2-associated cytokines IL-4, IL-5 and IL-13, as well as the pre-inflammatory cytokines IL-1 β and IL-6 in asthma have been well demonstrated [41,42]. Recently, Th17-associated cytokine IL-17 has been proven to have a role in asthma [43,44]. As a transcription factor, NF- κ B is involved in the regulation of the productions of IL-1 β , IL-6, IL-4, IL-5, IL-13 and IL-17 [8,10,11].

Consistent with the over-activation of NF- κ B, our results revealed that the OVA-treated rats had significantly increased levels of IL-1 β , IL-4, IL-5, IL-6, IL-13 and IL-17 in BALF. The changes in levels of the cytokines we observed in this study were similar to other previous studies [41–44]. However, berberine treatment dose-dependently inhibited the productions of these cytokines in the asthmatic rats. This is in line with the studies that reported berberine could regulate the cytokine production in other diseases. Based on the important role of the cytokines in asthma, we suppose that the attenuation of the cytokine production by berberine should be beneficial for the amelioration of asthma.

To confirm the therapeutic effects of berberine on the OVA-induced airway inflammation, we assessed the number of the inflammatory cells in BALF. Our results showed that the OVA-treated rats had increased number of eosinophils, macrophages,

neutrophil and lymphocytes in BALF. But berberine treatment dose-dependently reduced the number of these cells, indicating the anti-inflammatory effects on berberine on the OVA-induced airway inflammation. NF- κ B is thought to be important for the infiltration and the function of these inflammatory cells. Delayed eosinophil apoptosis in asthma is a major cause of prolonged eosinophil inflammation [45].

NF- κ B is thought to be important in eosinophil survival because the inhibition of NF- κ B in humans and animals induces eosinophil apoptosis [46,47]. NF- κ B also determines the productions of IL-4, IL-5 and IL-13 from Th2 cells, which stimulate B lymphocyte IgE production [48]. In addition, the secretion of numerous cytokines by neutrophils and alveolar macrophages involves NF- κ B activation [49,50]. Thus, the decrease in the number of inflammatory cells and the inhibition of NF- κ B signaling by berberine would be coordinately beneficial for asthma amelioration. Additionally, our histological examination also showed berberine treatment attenuated the OVA-induced lung inflammation in a dose-dependent manner in the asthmatic rats. Besides our findings, two recent studies also demonstrated that berberine ameliorated the cigarette smoke-induced lung inflammation in mice [23,24].

IgE is critical for allergic immune reaction. IL-4 is required for B cell maturation and IgE synthesis [51]. In line with the changes of IL-4, OVA-challenge resulted in very high serum levels of OVA-specific IgE in rats. Accompanied by the reductions in IL-4 production and NF- κ B activation, levels of OVA-specific IgE significantly decreased after berberine treatment.

Collectively, we conclude that berberine can attenuate the OVA-induced airway inflammation and inhibit the over-activation of the NF- κ B signaling pathway in Wistar rats. But, the pathophysiological changes of asthma are quite complex. This study only studied the anti-inflammatory effects of berberine in asthmatic animals. As is known, NF- κ B signaling is also associated with hyperresponsiveness, airway remodeling and other aspects of asthma. Whether berberine would exhibit anti-hyperresponsiveness activity, anti-remodeling activity and other effects through inhibiting NF- κ B signaling in asthma requires further study.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Funding

This work was supported by grants from Shandong Provincial Natural Science Foundation (ZR2013CM033, ZR2015CL029) and Shandong Provincial Education Department (J15LL54).

References

- Dong F, Wang C, Duan J, Zhang W, Xiang D, Li M. Puerarin attenuates ovalbumin-induced lung inflammation and hemostatic imbalance in rat asthma model. *Evid Based Complement Alternat Med* 2014;2014:726740.
- Durham A, Adcock IM, Tliba O. Steroid resistance in severe asthma: current mechanisms and future treatment. *Curr Pharm Des* 2011;17:674–684.
- Walsh GM. Anti-IL-4/-13 based therapy in asthma. *Expert Opin Emerg Drugs* 2015;20:349–352.
- Lindén A, Dahlén B. Interleukin-17 cytokine signalling in patients with asthma. *Eur Respir J* 2014;44:1319–1331.
- Charrad R, Berraies A, Hamdi B, Ammar J, Hamzaoui K, Hamzaoui A. Anti-inflammatory activity of IL-37 in asthmatic children: Correlation with inflammatory cytokines TNF- α , IL- β , IL-6 and IL-17A. *Immunobiology* 2016;221:182–187.
- Walsh GM. Novel cytokine-directed therapies for asthma. *Discov Med* 2011;11:283–291.
- Michael Schuliga. NF- κ B signaling in chronic inflammatory airway disease. *Biomolecules* 2015;5:1266–1283.
- Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A. A critical role for NF- κ B in GATA3 expression and TH2 differentiation in allergic airway inflammation. *Nat Immunol* 2001;2:45–50.
- Jung MY, Kim HS, Hong HJ, Youn BS, Kim TS. Adiponectin induces dendritic cell activation via PLC γ /JNK/NF- κ B pathways, leading to Th1 and Th17 polarization. *J Immunol* 2012;188:2592–2601.
- Min HK, Kim SM, Baek SY, Woo JW, Park JS, Cho ML, Lee J, Kwok SK, Kim SW, Park SH. Anthocyanin extracted from black soybean seed coats prevents autoimmune arthritis by suppressing the development of Th17 cells and synthesis of proinflammatory cytokines by such cells, via inhibition of NF- κ B. *PLoS One* 2015;10:e0138201.
- Zhang T, Yang S, Du J, Jinfu Y, Shumin W. Platycodin D attenuates airway inflammation in a mouse model of allergic asthma by regulation NF- κ B pathway. *inflammation* 2015;38:1221–1228.
- Shi H, Ma J, Mi C, Li J, Wang F, Lee JJ, Jin X. Amorphin A inhibits TNF- α -induced NF- κ B activation and NF- κ B-regulated target gene products. *Int Immunopharmacol* 2014;21:56–62.
- Sønder SU, Saret S, Tang W, Sturdevant DE, Porcella SF, Siebenlist U. IL-17-induced NF- κ B activation via CIKS/Act1: physiologic significance and signaling mechanisms. *J Biol Chem* 2011;286:12881–12890.
- Lai G, Wu C, Hong J, Song Y. 1,25-Dihydroxyvitamin D(3) (1,25-(OH)(2)D(3)) attenuates airway remodeling in a murine model of chronic asthma. *J Asthma* 2013;50:133–140.
- Gagliardo R, Chanez P, Mathieu M, Bruno A, Costanzo G, Gougat C, Vachier I, Bousquet J, Bonsignore G, Vignola AM. Persistent activation of nuclear factor- κ B signaling pathway in severe uncontrolled asthma. *Am J Respir Crit Care Med* 2003;168:1190–1198.
- Janssen-Heininger YM, Poynter ME, Aesif SW, Pantano C, Ather JL, Reynaert NL, Ckless K, Anathy V, van der Velden J, Irvin CG, van der Vliet A. Nuclear factor κ B, airway epithelium, and asthma: avenues for redox control. *Proc Am Thorac Soc* 2009;6:249–255.
- Guang Hai Yan, Yun Ho Choi. Phellinus linteus extract exerts anti-asthmatic effects by suppressing NF- κ B and p38 MAPK activity in an OVA-induced mouse model of asthma. *Immune Netw* 2014;14:107–115.
- Lin BF, Chiang BL, Ma Y, Lin JY, Chen ML. Traditional herbal medicine and allergic asthma. *Evid Based Complement Alternat Med* 2015;2015:510989.
- Chen J, Zhou H, Wang J, Zhang B, Liu F, Huang J, Li J, Lin J, Bai J, Liu R. Therapeutic effects of resveratrol in a mouse model of HDM-induced allergic asthma. *Int Immunopharmacol* 2015;25:43–48.
- Dong SF, Yasui N, Negishb H, Kishimoto A, Sun JN, Ikeda K. Increased oxidative stress in cultured 3T3-L1 cells was attenuated by berberine treatment. *Nat Prod Commun* 2015;10:895–897.
- Dai P, Wang J, Lin L, Zhang Y, Wang Z. Renoprotective effects of berberine as adjuvant therapy for hypertensive patients with type 2 diabetes mellitus: Evaluation via biochemical markers and color Doppler ultrasonography. *Exp Ther Med* 2015;10:869–876.
- Lee CH, Chen JC, Hsiang CY, Wu SL, Wu HC, Ho TY. Berberine suppresses inflammatory agents-induced interleukin-1 β and tumor necrosis factor- α productions via the inhibition of I κ B degradation in human lung cells. *Pharmacol Res* 2007;56:193–201.
- Lin K, Liu S, Shen Y, Li Q. Berberine attenuates cigarette smoke-induced acute lung inflammation. *Inflammation* 2013;36:1079–1086.
- Xu D, Wan C, Wang T, Tian P, Li D, Wu Y, Fan S, Chen L, Shen Y, Wen F. Berberine attenuates cigarette smoke-induced airway inflammation and mucus hypersecretion in mice. *Int J Clin Exp Med* 2015;8:8641–8647.
- Wang W, Zhu R, Xie Q, Li A, Xiao Y, Li K, Liu H, Cui D, Chen Y, Wang S. Enhanced bioavailability and efficiency of curcumin for the

- treatment of asthma by its formulation in solid lipid nanoparticles. *Int J Nanomed* 2012;7:3667–3677.
26. Yang YG, Tian WM, Zhang H, Li M, Shang YX. Nerve growth factor exacerbates allergic lung inflammation and airway remodeling in rat model of chronic asthma. *Exp Ther Med* 2013;6:1251–1258.
 27. Gueders MM, Balbin M, Rocks N, Foidart JM, Gosset P, Louis R, Shapiro S, Lopez-Otin C, Noël A, Cataldo DD. Matrix metalloproteinase-8 deficiency promotes granulocytic allergen-induced airway inflammation. *J Immunol* 2005;175:2589–2597.
 28. Bhattacharyya S1, Xue L, Devkota S, Chang E, Morris S, Tobacman JK. Carrageenan-induced colonic inflammation is reduced in Bcl10 null mice and increased in IL-10-deficient mice. *Mediators Inflamm* 2013;2013:397642.
 29. Punsawad C, Krudsood S, Maneerat Y, Chaisri U, Tangpukdee N, Pongponratn E, Nantavisai K, Udomsangpetch R, Viriyavejakul P. Activation of nuclear factor κ B in peripheral blood mononuclear cells from malaria patients. *Malar J* 2012;11:191.
 30. Löwhagen O. Diagnosis of asthma - new theories. *J Asthma* 2015;52:538–544.
 31. Fajt ML, Wenzel SE. Biologic therapy in asthma: entering the new age of personalized medicine. *J Asthma* 2014;51:669–676.
 32. Wang Z, Chen Z, Yang S, Wang Y, Huang Z, Gao J, Tu S, Rao Z. Berberine ameliorates collagen-induced arthritis in rats associated with anti-inflammatory and anti-angiogenic effects. *Inflammation* 2014;37:1789–1798.
 33. Ghosh S, May MJ, Kopp EB. NF- κ B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 1998;16:225–260.
 34. Hinz M, Scheidereit C. The I κ B kinase complex in NF- κ B regulation and beyond. *EMBO Rep* 2014;15:46–61.
 35. Schuliga M. NF- κ B signaling in chronic inflammatory airway disease. *Biomolecules* 2015;5:1266–1283.
 36. Kurakula K, Vos M, Logiantara A, Roelofs JJ, Nieuwenhuis MA, Koppelman GH, Postma DS, van Rijt LS, de Vries CJ. Nuclear receptor Nur77 attenuates airway inflammation in mice by suppressing NF- κ B activity in lung epithelial cells. *J Immunol* 2015;195:1388–1398.
 37. Zhou E, Fu Y, Wei Z, Yang Z. Inhibition of allergic airway inflammation through the blockage of NF- κ B activation by ellagic acid in an ovalbumin-induced mouse asthma model. *Food Funct* 2014;5:2106–2112.
 38. Wu MY, Hung SK, Fu SL. Immunosuppressive effects of fisetin in ovalbumin-induced asthma through inhibition of NF- κ B activity. *J Agric Food Chem* 2011;59:10496–10504.
 39. Liu W, Zhang X, Liu P, Shen X, Lan T, Li W, Jiang Q, Xie X, Huang H. Effects of berberine on matrix accumulation and NF- κ B signal pathway in alloxan-induced diabetic mice with renal injury. *Eur J Pharmacol* 2010;638:150–155.
 40. Fu K, Lv X, Li W, Wang Y, Li H, Tian W, Cao R. Berberine hydrochloride attenuates lipopolysaccharide-induced endometritis in mice by suppressing activation of NF- κ B signal pathway. *Int Immunopharmacol* 2015;24:128–132.
 41. Holt PG, Sly PD. Th2 cytokines in the asthma late-phase response. *Lancet* 2007;370:1396–1398.
 42. Sarinho ES, Azoubel-Antunes A, Rêgo MJ, Brayner-Cavalcanti M, Lins E, Lins TU, Pitta Ida R, Pitta MG. Evaluation of Th17-related cytokines and IFN γ production from blood mononuclear cells of moderate and severe asthmatic children reveals methylprednisolone does not decrease IL-22 levels. *J Asthma* 2015;52:227–231.
 43. Al-Ramli W, Préfontaine D, Chouiali F, Martin JG, Olivenstein R, Lemièrre C, Hamid QT(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J Allergy Clin Immunol* 2009;123:1185–1187.
 44. Bajoriuniene I, Malakauskas K, Lavinskiene S, Jeroch J, Sakalauskas R. Th17 response to dermatophagoides pteronyssinus is related to late-phase airway and systemic inflammation in allergic asthma. *Int Immunopharmacol* 2013;17:1020–1027.
 45. Kankaanranta H, Lindsay MA, Gienbycz MA, Zhang X, Moilanen E, Barnes PJ. Delayed eosinophil apoptosis in asthma. *J Allergy Clin Immunol* 2000;106:77–83.
 46. Park YM, Bochner BS. Eosinophil survival and apoptosis in health and disease. *Allergy Asthma Immunol Res* 2010;2:87–101.
 47. Fujihara S, Jaffray E, Farrow SN, Rossi AG, Haslett C, Hay RT. Inhibition of NF- κ B by a cell permeable form of I κ B alpha induces apoptosis in eosinophils. *Biochem Biophys Res Commun* 2005;326:632–637.
 48. Pelaia G, Vatrella A, Busceti MT, Gallelli L, Calabrese C, Terracciano R, Maselli R. Cellular mechanisms underlying eosinophilic and neutrophilic airway inflammation in asthma. *Mediators Inflamm* 2015;2015:879783.
 49. Ventura I, Vega A, Chamorro C, Aroca R, Gómez E, Pineda F, Palacios R, Blanca M, Monteseirín J. Allergen immunotherapy decreases LPS-induced NF- κ B activation in neutrophils from allergic patients. *Pediatr Allergy Immunol* 2014;25:129–135.
 50. Murugan V, Peck MJ. Signal transduction pathways linking the activation of alveolar macrophages with the recruitment of neutrophils to lungs in chronic obstructive pulmonary disease. *Exp Lung Res* 2009;35:439–485.
 51. Igarashi H, Kuwahara K, Yoshida M, Xing Y, Maeda K, Nakajima K, Sakaguchi N. GANP suppresses the arginine methyltransferase PRMT5 regulating IL-4-mediated STAT6-signaling to IgE production in B cells. *Mol Immunol* 2009;46:1031–1041.