

Bufalin Inhibits the Inflammatory Effects in Asthmatic Mice through the Suppression of Nuclear Factor-Kappa B Activity

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Key Words

Bufalin · Asthma · Ovalbumin · Inflammation · Nuclear factor-kappa B

Abstract

Asthma is an inflammatory airway disease characterized by increased infiltration of inflammatory cells into the airways and poor respiratory function. Bufalin is one of the biological ingredients obtained from *Chansu*. Bufalin was found to possess various pharmacological properties including anti-inflammatory activities. However, the effect of bufalin treatment on asthma has not yet been reported. Therefore, this study aimed to investigate the inhibitory effect of bufalin on asthmatic response in a murine model. A mouse asthma model was developed by ovalbumin (OVA) sensitization and challenge in the BALB/c mice. OVA-specific serum IgE and the levels of interleukin (IL)-4, IL-5, and IL-13 in bronchoalveolar lavage fluid (BALF) were determined by an enzyme-linked immunosorbent assay. Recruitment of inflammatory cells into BALF or lung tissues, and goblet cell hyperplasia were evaluated by histological staining. The expression levels of inhibitory subunit of nuclear factor-kappa B (NF-κB) alpha (IκBα) and phosphorylated p65 protein were measured by Western blot analyses. The results demonstrated that bufalin (5 and 10 mg/kg) markedly attenuated hyper-responsiveness, and strongly suppressed the OVA-induced

increases of total inflammatory cells including macrophages, eosinophils, lymphocytes, and neutrophils in BALF. The levels of IL-4, IL-5, and IL-13 in BALF and OVA-specific IgE in serum were significantly reduced by bufalin. Histological staining of lung tissues showed that bufalin reduced inflammatory cell infiltration and goblet cell hyperplasia. The results of Western blotting indicated that bufalin suppressed the IκBα degradation from NF-κB, and reduced the level of phosphorylated p65 protein in the lung tissues. These data suggest that bufalin can exert its anti-inflammatory effects possibly through the inhibition of the NF-κB activity.

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Introduction

Asthma is a chronic inflammatory lung disease characterized by variable airflow obstruction, airway hyper-responsiveness (AHR), chronic airway inflammation, and structural remodeling [1, 2]. Previous studies have indicated that antigen-specific T-helper lymphocytes (Th2 cells) and their cytokines orchestrate the pathological characteristics of asthma [3]. Exposure to aeroallergens in susceptible individuals results in Th2 cells re-

Z.Z. and M.H. contributed equally to this work.

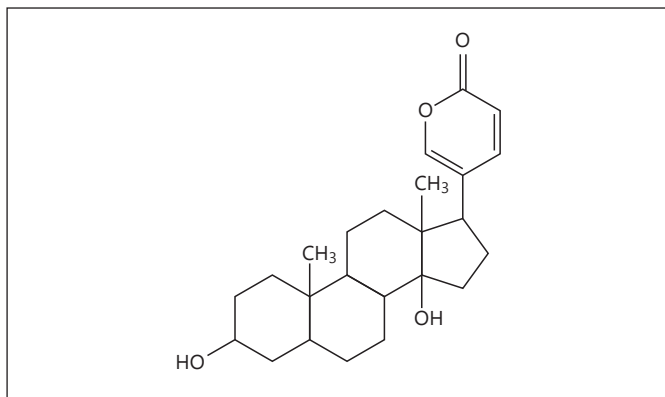


Fig. 1. The chemical structure of bufalin.

sponse and the release of allergen-specific IgE in serum, and accompanied by intrapulmonary production of interleukin (IL)-4, IL-5, and IL-13 by Th2 cells [4]. Now, asthma is one of the most common respiratory diseases in children and adults, and its incidence has apparently increased worldwide, and affects about 300 million people in the world [5, 6]. Although the advances in understanding its pathogenesis have been made, there is still no cure for this disease [7]. Inhaled corticosteroids (ICS) are widely used to be a frontline therapy for treating allergic asthma, but subsets of asthmatic patients exhibited a decreased sensitivity to these drugs, and long-term treatment has resulted in numerous side effects [8]. Therefore, the development of novel anti-inflammatory agents is needed for the treatment of asthma.

Bufalin is a major digoxin-like component of a traditional Chinese medicine *Chansu*, and it is obtained from the skin and parotid venom glands of the toad [9]. Bufalin is a cardiotonic steroid, and the chemical structure of bufalin is shown in Figure 1. Increasing studies have indicated that bufalin possesses a variety of pharmaceutical activities, such as cardiotonic, anesthetic, blood pressure stimulation, respiratory excitation, and anti-tumor activities [10, 11].

Chansu has been used to treat inflammatory diseases including tonsillitis and sore throat for thousands of years in China [12]. In recent years, bufalin was found to exert anti-inflammatory and analgesic effects in the carrageenan-induced paw edema of rodents [13]. To our knowledge, the possible benefits of bufalin in asthma treatment are still unavailable. Thus, in this study, we focused on investigating whether bufalin had a distinct anti-inflammatory effect in a murine model of allergic asthma.

Materials and Methods

Chemical and Antibodies

Bufalin was obtained from Sigma-Aldrich Biotechnology (St. Louis, MO, USA), dissolved in dimethylsulfoxide (DMSO) as 500 $\mu\text{mol/L}$ stock solution, which was stored at 4°C, and was diluted to the final used concentration with sterile phosphate buffer saline (PBS) immediately before use. The final concentration of DMSO in each sample was kept less than 0.01% (v/v), and had no apparent effect on experimental data in this study. Primary antibodies against the inhibitory subunit of nuclear factor-kappa B (NF- κB) alpha (I $\kappa\text{B}\alpha$), phospho-p65, and β -actin were purchased from the Cell Signaling Technology Company (Beverly, MA, USA).

Animal

Female BALB/c mice (6–8 weeks of age and weighing 18–22 g each) were purchased from Medical Experimental Animal Center of Guangdong Province (Guangzhou, China), and maintained in a pathogen-free animal room with a 12 h dark/light cycle. Mice were fed with standard chow ad libitum and water. All animal experiments were performed in accordance with the Guidelines of Animal Care and Use Committee of the First Affiliated Hospital of Xinjiang Medical University in this study.

Ovalbumin Sensitization and Challenge Protocols

Mice were randomly divided into 6 groups (7 mice/each group): control, ovalbumin (OVA), OVA + Dex (dexamethasone, 3 mg/kg, Sigma-Aldrich), OVA + Buf (bufalin 5 mg/kg), OVA + Buf (bufalin 10 mg/kg), and OVA + Bay 11-7085 (10 mg/kg, Sigma-Aldrich) that is an NF- κB inhibitor. Based on our previous preliminary experiments (data not shown), the doses of bufalin, 5 and 10 mg/kg, were used in this study. The mice were sensitized on days 0 and 14 by intraperitoneal injection (i.p.) of 20 μg OVA (Grade V, Sigma-Aldrich) adsorbed in 100 $\mu\text{g/mL}$ of Inject Alum (Pierce, Rockford, USA), followed by challenge with an aerosol of 1% OVA (wt/vol) in 0.9% saline for 30 min on days 22, 23, and 24. Control mice were sensitized and challenged using the same protocol but using only saline. Animals received bufalin i.p. (5 and 10 mg/kg) on days 22–24 starting 1 h before each OVA challenge. Bay 11-7085 as an NF- κB inhibitor was dissolved in sterile DMSO-PBS, and injected intraperitoneally into the animals on days 22–24 starting 1 h before each OVA provocation. Dex was used as a positive control, and administered i.p. in the same manner. The vehicle (less than 0.01% v/v DMSO) had no significant effect on the data in this study (data not shown).

Measurement of AHR

AHR was evaluated as described previously [14]. Briefly, the mice were anaesthetized with sodium pentobarbital (60 mg/kg) by an i.p. injection 24 h after the final OVA challenge, and were inserted with a plastic tube (2 mm internal diameter) into the trachea through tracheotomy, followed by bufalin, dexamethasone, or NF- κB inhibitor (Bay 11-7085) administration via the caudal vein with a 27-gauge needle. Methacholine in increasing concentrations (from 6.25 to 50 mg/mL) was separately administered intravenously. Airway resistance, which refers to the pressure-driving respiration divided by flow, was measured by a whole-body plethysmographic chamber (Catalog = # PLY3213, Buxco Electronics Inc., NY, USA) as described according to the manufactur-

er's manual, and expressed as Penh (enhanced pause). The results are expressed as the ratio of the respective baseline values obtained from the control mice.

Analysis of Bronchoalveolar Lavage Fluid

The mice were sacrificed 24 h after the final challenge, and thoracic cavities of mice were immediately opened, and bronchoalveolar lavage fluid (BALF) was collected by cannulating the upper part of the trachea and lavaging with PBS in 3 aliquots (0.4 mL/each). Subsequently, lavaged samples were centrifuged at 400 g for 5 min at 4°C and the cells pellets were resuspended in 500 µL of PBS for analysis of a total number of inflammatory cells in BALF with a hemocytometer. Smears of BALF cells were performed as described previously [15] and stained with a modified Wright's staining for the differential cell counts that were performed on at least 300 cells in each slide according to standard morphological criteria.

Enzyme-Linked Immunosorbent Assay

Blood samples were immediately collected from the retro-orbital plexus after sacrifice, and then centrifuged at 800 g for 15 min. For OVA-specific serum IgE analysis, aliquots of serum were detected by using an enzyme-linked immunosorbent assay (ELISA) kit (BioLegend, CA, USA) according to the manufacturer's instructions. The levels of IL-4, IL-5, and IL-13 in BALF were assayed by specific mouse IL-4, IL-5, and IL-13 ELISA kits (BD Bioscience) following the manufacturer's instructions.

Lung Histology

Lung tissue samples were obtained from the sacrificed mice, and fixed in 4% paraformaldehyde, and embedded in paraffin, and sectioned at 5 µm. Subsequently, the sections were stained with hematoxylin and eosin (H&E) to detect the infiltration of inflammatory cells, and periodic acid-Schiff (PAS) to evaluate airway goblet cells. To evaluate the extent of mucus production, the mucus score was quantified as previously described [16]. A 5-point grading system was performed by a blinded scorer: 0, no goblet cells; 1, <25%; 2, 25–50%; 3, 51–75%; 4, >75%. Mucus score was performed in at least 4 different fields for each lung section.

Western Blot Analysis

Cell extractions were obtained from fresh lung tissues using lysis buffer-added protease inhibitors and phosphatase inhibitors. After the determination of protein concentrations, equal amounts (15 µg) of total protein were loaded on a 10% SDS-PAGE gel, and then transferred to polyvinylidene difluoride membranes (Millipore, USA). After blocking in 5% nonfat milk in TBST buffer for 2 h, the membranes were incubated with anti-IκBα (1:1,000 dilution, Catalog = #4812, Cell Signal Technology), anti-phosphorylated p65 (1:500 dilution, Catalog = #3033, Cell Signal Technology), and anti-β-actin (1:1,000 dilution, Catalog = #4970, Cell Signal Technology) antibodies overnight at 4°C, followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (1:1,000 dilution, Catalog = #ab6721, abcam) for 1 h at room temperature. Protein bands were then detected on an X-ray film using an enhanced chemiluminescence detection system (Amersham Biosciences Corp., NJ, USA). Quantity One software (Bio-Rad, Hercules, CA, USA) was used to quantify the optical density of each band signal. The experiments were independently performed in triplicate.

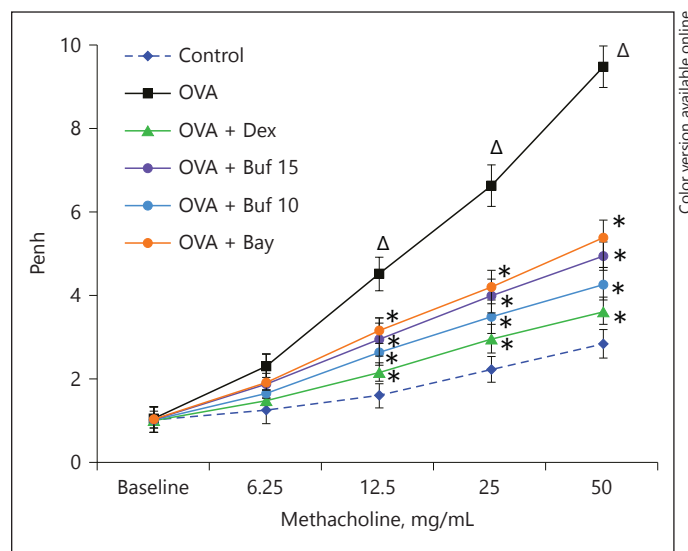


Fig. 2. Bufalin alleviates the development of AHR in a mouse model of asthma. AHR was measured using a whole-body plethysmography after airway exposure to increased concentrations of methacholine as described in the Materials and Methods. Data are expressed as means $\pm$ SEM ($n = 7$). OVA + Dex, OVA-challenged mice administered Dex; OVA + Buf 5, OVA-challenged mice administered bufalin (5 mg/kg); OVA + Buf 10, OVA-challenged mice administered bufalin (10 mg/kg); OVA + Bay, OVA-challenged mice administered Bay 11-7085. $\Delta p < 0.05$ vs. control; $* p < 0.05$ vs. OVA.

Statistical Analyses

All the data were expressed as the mean $\pm$ SEM. Statistical analyses were performed using SPSS 13.0 statistical software, and group comparisons were performed with one-way analysis of variance. $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Effect of Bufalin on AHR

To investigate the effect of bufalin on AHR in response to increased concentrations of methacholine, airway resistance of mice was determined in Penh, and the results demonstrated that no significant differences in baseline airway resistance were observed among the 6 groups. Methacholine administration from 6.25 to 50 mg/mL resulted in a dramatic increase of airway resistance in the OVA-challenged mice when compared with the control mice. Bufalin treatment (5 or 10 mg/kg) dramatically prevented AHR in response to methacholine (Fig. 2). The treatment with dexamethasone, or with NF-κB inhibitor (Bay 11-7085), also resulted in similar effects on AHR. These findings suggest that bufalin can reduce airway hyperreactivity and alleviate airway pathology-mediated inflammatory response in vivo.

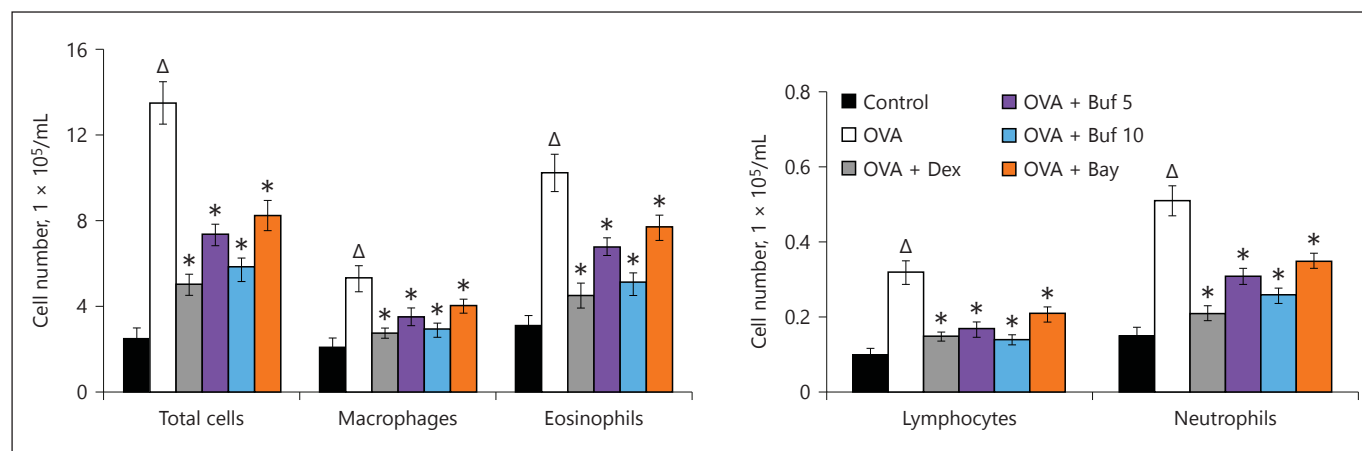


Fig. 3. Bufalin treatment suppresses inflammatory cell accumulation in BALF in a mouse model of asthma. Cell counts were measured as described in the Materials and Methods. Data are expressed as means $\pm$ SEM ($n = 7$). OVA + Dex, OVA-challenged mice administered Dex; OVA + Buf 5, OVA-challenged mice administered bufalin (5 mg/kg); OVA + Buf 10, OVA-challenged mice administered bufalin (10 mg/kg); OVA + Bay, OVA-challenged mice administered Bay 11-7085. ^Δ $p < 0.05$ vs. control; * $p < 0.05$ vs. OVA.

Bufalin Attenuates Allergic Airway Inflammation

To evaluate the anti-inflammatory effect of bufalin, the total cell counts and differential cell counts in the BALF of mice were determined 24 h after the final OVA challenge. The results showed that OVA challenge resulted in a marked increase of total leukocytes, macrophages, eosinophils, lymphocytes, and neutrophils in the BALF compared with the control mice. However, in comparison with the OVA-challenged mice, bufalin treatment significantly reduced the amounts of these inflammatory cells. The treatment with dexamethasone or NF- κ B inhibitor (Bay 11-7085) also produced similar inhibitory effects on these inflammatory cells (Fig. 3).

The sections of lung tissue were evaluated by H&E staining for further analysis of the anti-inflammatory effect of bufalin. Results showed that no inflammatory cell infiltration was observed in the normal lung parenchyma of the control mice (Fig. 4a). But an obvious inflammatory cell infiltration in the peribronchial and perivascular areas of the lung tissue was shown in the OVA-challenged mice (Fig. 4b). However, the administration of dexamethasone or bufalin (10 mg/kg) markedly attenuated inflammatory cell infiltration compared with the OVA-challenged mice (Fig. 4c, d). On the other hand, lung tissue sections stained with PAS demonstrated no signs of mucus or goblet cell hyperplasia in the control mice (Fig. 4e), but OVA-challenged mice developed apparent goblet cell hyperplasia and mucus hypersecretion in the airways (Fig. 4f). The treatment with dexamethasone or bufalin (10 mg/kg) markedly reduced the num-

ber of PAS-stained goblet cells and epithelial cell disruption in the airways (Fig. 4g, h). Further analyses exhibited a significant decrease of mucus score in the mice treated with dexamethasone, bufalin (5 and 10 mg/kg) or Bay 11-7085 when compared with that in the OVA-challenged mice (Fig. 4i), which suggested that OVA-induced mucus hypersecretion was markedly halted by bufalin (5 and 10 mg/kg).

Bufalin Reduces the Levels of IL-4, IL-5, IL-13 in BALF and OVA-Specific IgE in Serum

The levels of Th2 cytokines in BALF and OVA-specific IgE in serum were determined by ELISA assays, and the results indicated that dramatic increases of IL-4, IL-5 and IL-13 in BALF, and IgE in serum were observed in the OVA-challenged mice compared with the control mice. In comparison with OVA-challenged mice, bufalin (5 or 10 mg/kg) treatment significantly reduced the levels of IL-4, IL-5, and IL-13 in BALF, and simultaneously lowered the level of OVA-specific IgE in serum. Pre-treatment with dexamethasone or NF- κ B inhibitor (Bay 11-7085) also resulted in similar inhibitory effects on IL-4, IL-5, IL-13, and OVA-specific IgE in the mice (Fig. 5).

Bufalin Inhibits NF- κ B Activity in OVA-Challenged Mice

It is well known that NF- κ B is a key transcription factor in the modulation of acute inflammatory response, and it plays a critical role in asthma by inducing the transcription of various proinflammatory mediators [17]. Our present

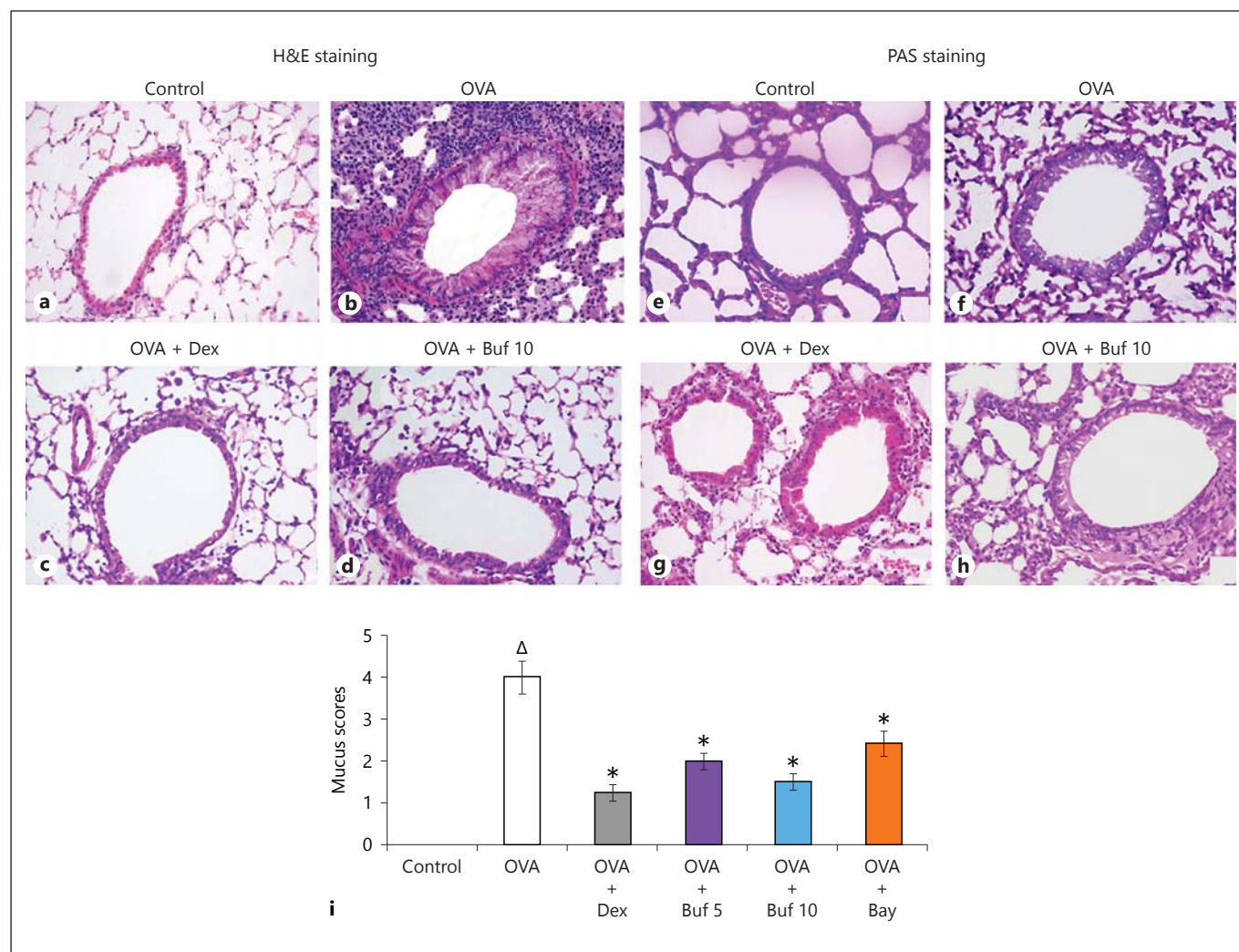


Fig. 4. Bufalin treatment inhibits the infiltration of inflammatory cells and goblet cell hyperplasia in lung tissue. **a–d** Representative images of H&E stained sections from lung tissues, magnification $\times 200$. **e–h** Representative images of PAS staining to analyze goblet cell hyperplasia of lung tissues, magnification $\times 200$. **i** Mucus scores. The data are expressed as means $\pm$ SEM ($n = 7$). OVA +

Dex, OVA-challenged mice administered Dex; OVA + Buf 5, OVA-challenged mice administered bufalin (5 mg/kg); OVA + Buf 10, OVA-challenged mice administered bufalin (10 mg/kg); OVA + Bay, OVA-challenged mice administered Bay 11-7085. ^Δ $p < 0.05$ vs. control; * $p < 0.05$ vs. OVA.

data demonstrated that eosinophilic inflammation in this asthmatic model of mice was blocked by a specific NF- κ B inhibitor (Bay 11-7085). Therefore, we hypothesized that the anti-inflammatory effects of bufalin involves in suppressing NF- κ B activation. To test this hypothesis, we detected the expression levels of I κ B α and phosphorylation of p65 subunit of NF- κ B. The results of Western blotting showed that the degradation of I κ B α and upregulation of p65 phosphorylated form were observed in the OVA-challenged mice. However, the administration of bufalin, dexamethasone, or NF- κ B inhibitor (Bay 11-7085) mark-

edly inhibited the I κ B α degradation and downregulated the level of p65 phosphorylation (Fig. 6). These findings suggest that bufalin may exert its anti-inflammatory effects through the inhibition of NF- κ B activity.

Discussion

Asthma is a chronic inflammatory disease in which Th2 cells and their cytokines, such as IL-4, IL-5, and IL-13, are critical to asthma pathobiology [18]. ICS are

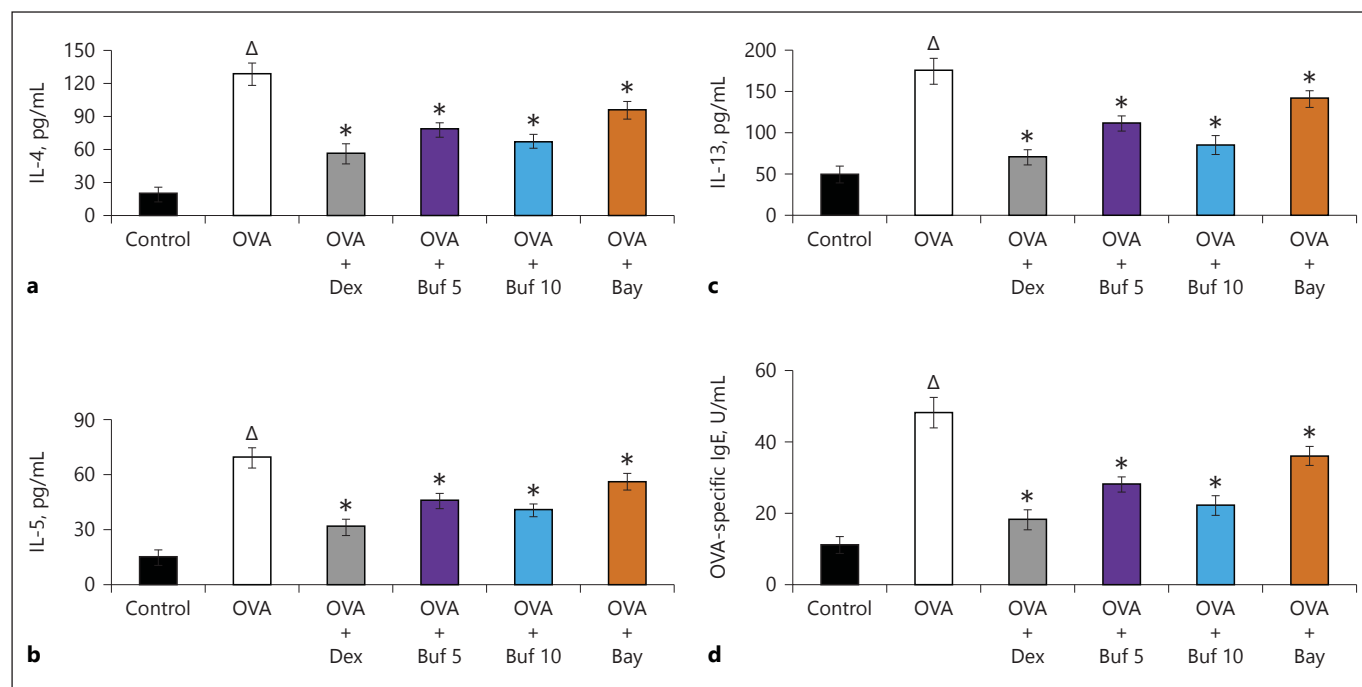


Fig. 5. Effects of bufalin on the levels of Th2 cytokines in BALF and OVA-specific IgE in serum. **a–d** The levels of IL-4 (**a**), IL-5 (**b**), IL-13 (**c**) in BALF and OVA-specific IgE (**d**) were determined by ELISA assays. The data are expressed as means $\pm$ SEM ($n = 7$). OVA + Dex, OVA-challenged mice administered Dex;

OVA + Buf 5, OVA-challenged mice administered bufalin (5 mg/kg); OVA + Buf 10, OVA-challenged mice administered bufalin (10 mg/kg); OVA + Bay, OVA-challenged mice administered Bay 11-7085. ^Δ $p < 0.05$ vs. control; * $p < 0.05$ vs. OVA.

the cornerstone of maintenance therapy for asthma, and have improved asthma-related symptoms and morbidity and mortality, but a high number of patients being treated according to guidelines remain difficult to control with frequent exacerbations and persisting symptoms [19]. Consequently, development of new agents is increasingly needed for its treatment. In this study, we have demonstrated that bufalin possesses an effective anti-inflammatory role in a murine model of asthma probably through inhibition of NF- κ B activity.

Bufalin is a major digoxin-like immunoreactive component of *Chansu*, a traditional Chinese medicine produced from the skin and parotid venom glands of the toad [20]. Bufalin is structurally similar to digitoxin (Fig. 1). Bufalin is a cardiotonic steroid that exhibits a variety of biological activities, such as cardiotonic, anesthetic, blood pressure stimulation, respiration, and anti-neoplastic activities [21]. Additionally, accumulating evidence has demonstrated that bufalin can exert anti-cancer effects in various types of human cancer cells, such as hepatocellular carcinoma, gastric, leukemia, and lung cancer cells [22, 23]. But very little is known about the anti-inflammatory effects of bufalin, although Chan

Chansu has been used in the treatment of inflammatory diseases, such as tonsillitis and sore throat, for thousands of years in China [12]. Recent studies have indicated that bufalin reduces tumor necrosis factor (TNF)- α -induced IL-1 β , IL-6, and IL-8 production in rheumatoid arthritis fibroblast-like synoviocytes, suggesting an anti-inflammatory role of bufalin in these cells [24]. These studies prompted our investigation of the use of bufalin in inflammatory diseases such as asthma. In the present study, our findings indicated that bufalin treatment abrogated OVA-induced airway inflammation, attenuated hyperresponsiveness and inflammation, and inhibited the release of IL-4, IL-5, IL-13, and OVA-specific IgE in the BALB/c mice. This is the first time that the anti-inflammatory effects of bufalin in a murine model of asthma are being evaluated.

Numerous studies have indicated that Th2 cells play an essential role in the initiation and progression of allergic asthma by the induction of cytokine secretion [25]. IL-4, IL-5, and IL-13 released by Th2 cells result in eosinophils-rich inflammation in lungs, and elevate the level of serum IgE and mucus hypersecretion by epithelial goblet cells [26]. In particular, IL-5 can promote the prolifera-

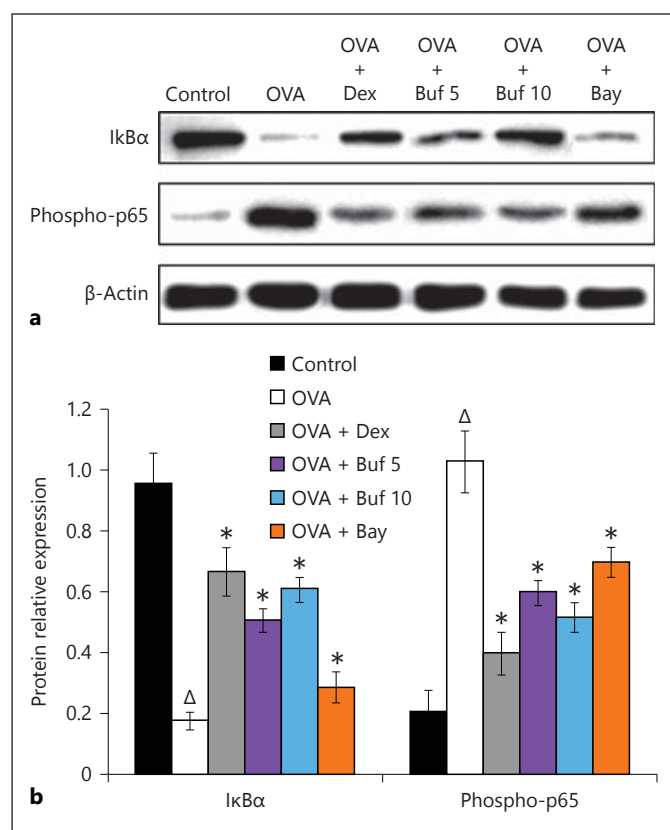


Fig. 6. Bufalin treatment suppresses NF- κ B activity in OVA-sensitized mice. Protein samples were prepared from lung tissues, and isolated on a 10% SDS-PAGE gel. **a** The representative images of Western blotting for the analyses of I κ B α and phospho-p65 protein expression. **b** The relative expression levels of I κ B α and phospho-p65 protein. β -Actin was used as the loading control. The data are expressed as means $\pm$ SEM ($n = 7$). OVA + Dex, OVA-challenged mice administered Dex; OVA + Buf 5, OVA-challenged mice administered bufalin (5 mg/kg); OVA + Buf 10, OVA-challenged mice administered bufalin (10 mg/kg); OVA + Bay, OVA-challenged mice administered Bay 11-7085. ^Δ $p < 0.05$ vs. control; * $p < 0.05$ vs. OVA.

tion and activation of eosinophils, and augment the infiltration of eosinophils into the airways [27]. In this study, our data showed that bufalin treatment significantly decreased the levels of IL-4, IL-5, and IL-13 in BALF in the OVA-challenged mice. Additionally, a downregulated level of OVA-specific serum IgE was observed in these sensitized mice after bufalin treatment. Further investigation indicated that bufalin strongly attenuated the increase of inflammatory cells such as eosinophils, macrophages, lymphocytes, and neutrophils. These findings were confirmed by subsequent histological staining, which demonstrated that bufalin treatment ameliorated the airway inflammatory response and inhibited inflam-

matory cell infiltration and mucus hypersecretion that was involved in airway obstruction [28]. Similar findings were observed in the dexamethasone or NF- κ B inhibitor (Bay 11-7085)-treated mice. These data demonstrate a strong anti-inflammatory effect of bufalin on the OVA-induced airway inflammation of mice.

Bufalin is one of cardiotonic steroids that induce diverse physiological effects on the heart muscle and blood pressure regulation, but the underlying mechanisms remain unknown, despite a long history of therapeutic applications and model studies [29]. However, several lines of evidence have indicated that bufalin can exhibit numerous biological activities possibly through different mechanisms, such as suppression of Na⁺/K⁺-ATPase [30], ploy (ADP-ribose) polymerase 1 (PARP1) [31], and Topo π [30], activation of AP-1 [32], and cdc2 kinase, and an increase of intracellular calcium concentrations [33]. Although the exact mechanism of bufalin-induced anti-inflammation remains unclear, the inhibition of NF- κ B activation may result in the anti-inflammatory action of bufalin in the murine asthmatic model. It is well known that NF- κ B is an important regulator in a broad spectrum of inflammatory networks that modulate cytokine activity in airway pathology [34], and it is also an essential transcription factor regulating the expression of specific genes in response to various environmental factors including allergens [35], and these genes encode various cytokines, chemokines, and receptors involved in immune recognition, and proteins participated in antigen presentation [36]. Increased activation of NF- κ B has been observed in the lung tissues after allergen challenge in airway smooth muscle cells, epithelial cells, and macrophages of asthmatic patients [37]. NF- κ B activation involves I κ B α phosphorylation catalyzed by I κ B kinase. NF- κ B activation will occur when it dissociates from I κ B α that is a negative regulator of NF- κ B [38]. In this study, our data demonstrated that bufalin treatment strongly suppressed the degradation of I κ B α from NF- κ B/I κ B α complex, suggesting that NF- κ B cannot translocate into the nucleus where it induces gene transcription by binding to the promoter of NF- κ B responsive genes [39]. Similar findings indicated that bufalin significantly inhibited the activation of NF- κ B in vivo by maintaining the level of I κ B α , and reduced the nuclear translocation of NF- κ B p65 [13]. It was reported that pretreatment with NF- κ B p65 antisense significantly inhibited the established asthmatic reaction in a murine model [40]. Our data demonstrated that bufalin treatment markedly reduced the level of p65 phosphorylation in the OVA-challenged mice, and pretreatment with an NF- κ B inhibitor (Bay 11-7085) produced similar inhibitory ef-

fects on inflammation responses with bufalin in OVA-challenged mice. These results suggest that bufalin may exert its anti-inflammation effects through inhibition of NF- κ B activity. Recent studies have demonstrated that bufalin also exhibits a strong, dose-dependent anti-inflammatory effect on carrageenan-induced paw edema in rats by suppressing the activation of NF- κ B and reducing the production of its downstream proinflammatory mediators during acute inflammation [13]. Additionally, the bufalin analogues, ouabain, and digoxin, also possess strong anti-inflammatory effects [41, 42]. In this study, dexamethasone was used as a reference drug, and it showed similar activities as that of bufalin. Dexamethasone is one of the corticosteroids that are the most potent anti-inflammatory agents used to treat chronic inflammatory diseases such as bronchial asthma. NF- κ B is a major target for corticosteroids that exert their effects by binding to glucocorticoid receptors, which are expressed in almost all cell types [43]. Previous studies have demonstrated that dexamethasone is able to inhibit AP-1- and NF- κ B-driven gene transcription. In this study, we did not investigate whether bufalin could bind to glucocorticoid receptors or inhibit AP-1. The interactions between activated glucocorticoid receptors and transcription factors, such as NF- κ B and AP-1, are important for corticosteroids to exert their anti-inflammatory effects. We speculate that inhibition of NF- κ B activity is possibly one of the common mechanisms of anti-inflammation for dexamethasone and bufalin. Various therapy strategies targeted NF- κ B signaling pathways such as proteasome inhibitors, IKK β -selective small molecule inhibitors, p65-specific antisense and small interfering ribonucleic acid, and NF- κ B-specific decoy oligonucleotide have demonstrated beneficial effects in experimental models of asthma [16, 44]. It is noted that we did not detect whether bufalin can affect p65 translocation from cytosol to nucleus, which is needed to be clarified in future research. Additionally, the proinflammatory mediators of NF- κ B downstream, such as cy-

cloxygenase-2, inducible nitric oxide synthase, IL-6, IL-1 β , and TNF should be determined in OVA-challenged animal in future work because of their correlation with the anti-inflammatory action of bufalin. On the other hand, systemic absorption of inhaled glucocorticoids at high doses may have deleterious effects. The potential risk or side-effects of bufalin needs to be widely investigated in future research before its clinical application for asthma treatment.

Conclusions

Our findings demonstrated that bufalin possessed a strong ability to reduce airway hyperreactivity, and attenuated inflammatory cells infiltration and hyperplasia of goblet cells to the airways in an OVA-induced murine model of asthma. Bufalin treatment significantly downregulated the levels of IL-4, IL-5, and IL-13 in BALF and OVA-specific IgE in serum, and inhibited the I κ B α degradation and p65 phosphorylation in the lung tissues of mice. These findings suggest that bufalin is a potential anti-inflammatory agent in asthma treatment at least through the suppression of NF- κ B activity.

Disclosure Statement

The authors declare that they have no competing interests.

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