



Bulleyaconitine A inhibits the lung inflammation and airway remodeling through restoring Th1/Th2 balance in asthmatic model mice

Liping Liu, Shuyun Wang*, Haiyan Xing, Yuemei Sun, Juan Ding and Ning He

Department of Allergy, Yantai Yuhuangding Hospital, Yantai, China

ABSTRACT

The current study aimed to study the effects of Bulleyaconitine A (BLA) on asthma. Asthmatic mice model was established by ovalbumin (OVA) stimulation, and the model mice were treated by BLA. After BLA treatment, the changes in lung and airway resistances, total and differential leukocytes in the bronchoalveolar lavage fluid (BALF) were detected, and the changes in lung inflammation and airway remodeling were observed. Moreover, the secretion of IgE, Th1/Th2-type and IL-17A cytokines in BALF and serum of the asthmatic mice were determined. The results showed that BLA attenuated OVA-induced lung and airway resistances, inhibited the inflammatory cell recruitment in BALF and the inflammation and airway remodeling of the asthmatic mice. In addition, BLA suppressed the secretion of IgE, Th2-type cytokines, and IL-17A, but enhanced secretions of Th1-type cytokines in BALF and serum. The current study discovered that BLA inhibited the lung inflammation and airway remodeling via restoring the Th1/Th2 balance in asthmatic mice.

ARTICLE HISTORY

Received 14 January 2020
Accepted 26 March 2020

KEYWORDS

Asthma; Bulleyaconitine A; inflammation; airway remodeling; Th1/Th2 cytokines

Asthma is a serious health problem that affects people of all ages all over the world [1], and the prevalence of asthma is still increasing [1,2]. Allergic asthma is characterized by reversible airway obstruction to allergens, chronic airway inflammation, and nonspecific airway hyperresponsiveness (AHR) [3]. Although asthma is considered as a condition of reversible airflow obstruction, many asthma patients still show residual airway obstruction [4], suggesting that irreversible component of airways obstruction is reflected in the presence of airway remodeling. Airway remodeling can further lead to airway dysfunction and is correlated to the onset of severe asthma [5]. The process of airway remodeling involves collagen deposition, sub-epithelial fibrosis, smooth muscle mass, wall thickening, and proliferation and angiogenesis of goblet cells [3,6]. Although the diagnosis and treatment of allergic asthma has been improved greatly than before, the disease still remains one of the most common chronic respiratory diseases that affect more than 10% of all population in the world [2,7]. At present, though glucocorticoids are widely used to treat asthma, but they could produce side effects and contributes to development of drug resistance of patients [8]. Based on these limitations, it is urgent to find effective methods to prevent and treat asthma.

Bulleyaconitine A (BLA) is a diterpenoid alkaloid isolated from *Aconitum bulleyanum* plants, which are widely used in the treatment of chronic pain in China [9]. BLA could relieve different types of chronic pains such as back pain, joint pain, and neuropathic pain with fewer

side effects, and the use of it as an anodyne has been permitted in clinical practice by China Food and Drug Administration since 1985 [10]. Researches have previously reported the mechanisms of BLA in chronic pain treatment: Zhu et al. found that BLA could inhibit presynaptic transmitter release to attenuate neuropathic pain [9]; Xie et al. showed that the mechanisms of BLA in anti-neuropathic pain may be realized through Na channels in uninjured neurons [11]; Li et al. demonstrated that the ability of BLA to activate the cAMP-PKA-p38 β -CREB pathway may be its potential anti-nociceptive mechanism [12]. From these reports, BLA potentially has an anti-hypersensitive effect [13]. In addition, it has been reported that BLA could inhibit lung inflammation in allergic asthma [14]. Moreover, BLA inhibits Itch and Itch Sensitization Induced by Histamine and Chloroquine [15], and attenuates hyperexcitability of dorsal root ganglion neurons induced by spared nerve injury [16]. However, the effects and mechanisms of BLA on asthma should be further investigated and confirmed.

Therefore, this study aimed to explore the effects of BLA on asthma and the underlying mechanisms in a murine asthmatic model.

Materials and methods

Ethics statement

All animal experiments were performed in accordance with the guidelines of the China Council on Animal Care and Use. This study was approved by the

Committee of Experimental Animals of Yantai Yuhuangding Hospital (Z20190105032 H). All possible efforts have been made in consideration of animal welfare. The animal experiments were performed in Yantai Yuhuangding Hospital.

Mice

Sixty adult female BALB/c mice (18–20 g, 6–8 weeks) were purchased from SLAC Laboratory Animal Technology (Shanghai, China). All the animals were fed in the same animal feeding unit under 12 h dark/12 h light cycle.

BLA

BLA, with a molecular weight of 627.76 and purity of higher than 98%, was purchased from Yuanye (107668-79-1, Shanghai, China), and stored at 4°C for later use. Before using, BLA was dissolved in 1 mL normal saline (R21480, Yuanye) and given to the mice at the prescribed dose.

Chronic asthmatic model establishment and treatment

The animals were randomly divided into four groups ($n = 15$), and stimulated by chronic ovalbumin (OVA) to establish an asthmatic model [4]. In brief, on day 0 and 7, the mice were sensitized by intravenous injection of 0.2 mL saline solution containing 20 μ g OVA (vacstova, Invivogen, Toulouse, France) and 2 mg aluminum hydroxide (HY-B1521, MedChem Express, Shanghai, China). The mice were exposed to 2% OVA solution for 30 min by ultrasonic nebulizer from day 14 on three times a week for 6 weeks (Figure 1(a)). Mice in the control group were only sensitized and treated by saline solution. From day 14 on, the mice in control and asthmatic (OVA) groups were orally administrated with saline solution, while those in Bulleyaconitine A (BLA) group were intraperitoneally given BLA (0.48 mg/kg). In tiotropium bromide (Tio) group, the mice received intranasal drip of 0.1 mm Tio (S61229, Yuanye) 30 min before the OVA stimulation and were considered as a positive drug group. After the completion of the program, the animals were anesthetized by intraperitoneal injection of overdose of sodium pentobarbital (R20830, Yuanye), and sacrificed by cervical dislocation method. Finally, the blood and lung tissues of the animals were obtained for subsequent analysis.

Assessment of AHR

AHR tests were performed 24 h after the OVA treatment of the mice by forced oscillation technique. In brief, the mice were anesthetized by intraperitoneal injection of sodium pentobarbital. Then a catheter

was quickly inserted into trachea, connected to the FlexiVent (SCIREQ, Montreal, PQ, Canada) and mechanically ventilated. Lung resistance was measured by forced oscillation technique. Airway responsiveness was determined by increasing doses of inhaled methacholine (MCh). Data were collected at 1-min interval, moreover, lung function values, including lung resistance ($\text{cm H}_2\text{O/mL/s}$) and airway resistance ($\text{cm H}_2\text{O/mL/s}$), were plotted to reflect the effects of different MCh doses.

Bronchoalveolar lavage fluid (BALF) cell collection

After the catheter was inserted into the trachea, 2 mL cold PBS (C0221A, Beyotime, Shanghai, China) was injected into the lung by syringe, which was then pulled back and forth twice. BALF samples were collected into 1.5 mL tube (615,001, Nest, Wuxi, China) and centrifuged at $4000 \times g$ for 10 min. Then the supernatants were stored at -80°C for cytokine measurement. The cell pellets of BAL were re-suspended in 200 μ L PBS. The cells were stained by trypan blue (C0011, Beyotime) to count the total cell number under a phase-contrast optical microscope (Axio Lab.A1 pol; Leica, Solms, Germany). Cell differentiation was determined by a Diff-Quick Stain Kit (40748ES60, YEASEN, Shanghai, China).

Histopathology

After we obtained the lung tissues from the sacrificed mice, the tissues were fixed with 4% paraformaldehyde (P804536, Macklin, Shanghai, China). The tissues were routinely processed, paraffin-embedded in (S25190, Yuanye), and sectioned into 5- μ m slices. Then the slices were stained by hematoxylin and eosin (H&E) (H-3502, Vectorlabs, MA, USA), periodic acid-Schiff (PAS) (395B, Sigma, CA, USA), and trichrome stain (Masson) (HT15-1KT, Sigma) for pathological observation of the lung tissues. Then the images were collected by Leica DM4000B microscope under a light microscope.

Cytokine measurement

Cytokines secretion levels of the supernatants of BALF and the mouse serums were analyzed by enzyme-linked immunosorbent assay (ELISA). The ELISA kit of mice IgE (DCM060-9), OVA-specific IgE (ml063583), IFN- γ (ml002277), IL-12 (ml037881), eotaxin (ml002213), IL-4 (ml064310), IL-5 (ml063157), IL-13 (ml063123), IL-33 (ml063153), and IL-17A (ml037864) were purchased from Enzyme-linked Biotechnology (Shanghai, China). In brief, the wells in a coated micro-well plate were filled with 50 μ L of each concentration of standard, or 25 μ L test samples with 25 μ L biotin. All the wells were filled

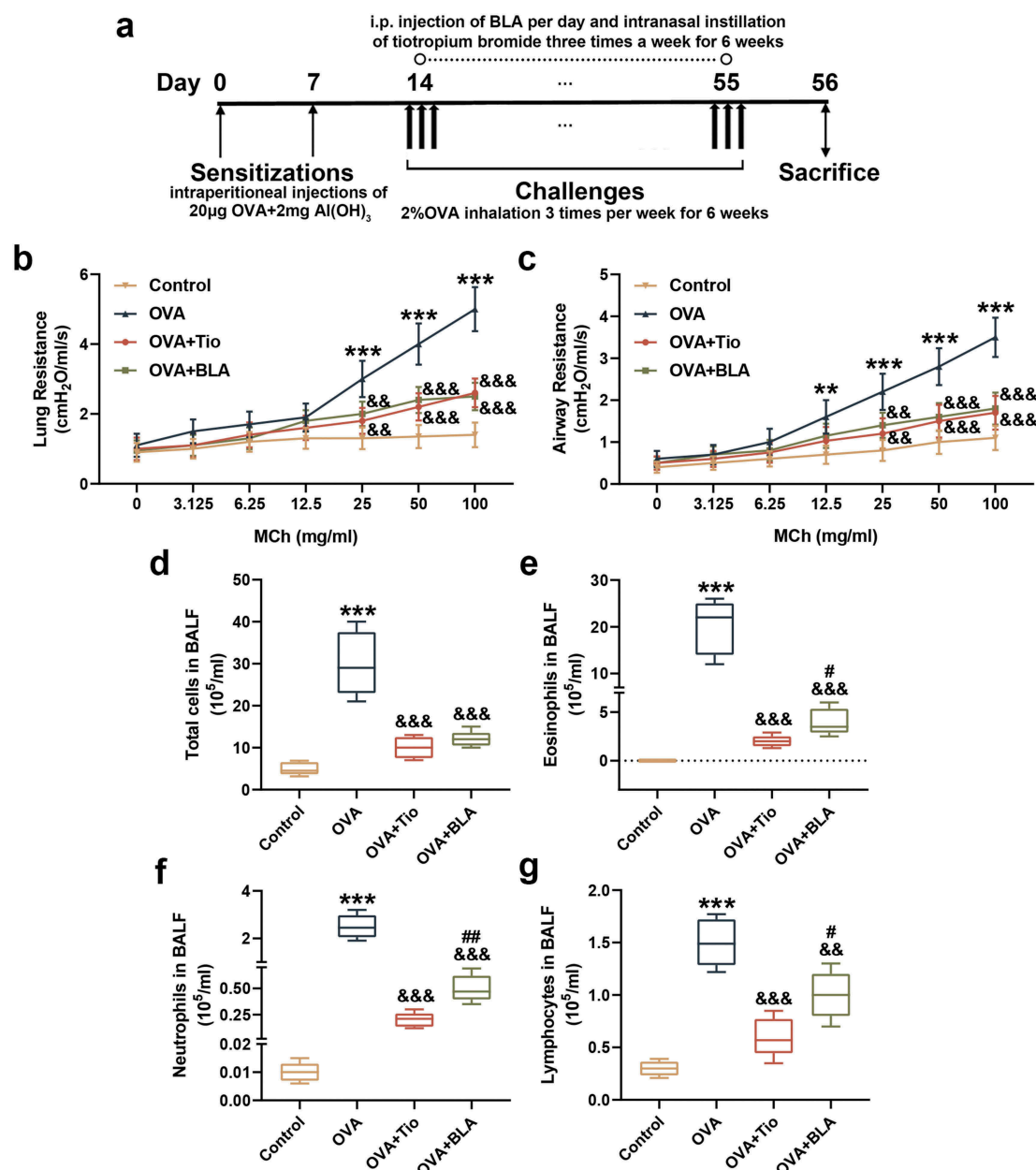


Figure 1. BLA attenuates AHR in chronic OVA-induced asthma and inhibits inflammatory cell recruitment in BALF. (a). Experimental layout to develop the model of chronic asthma in mice. (b, c). Lung resistance and airway resistance in BALF were detected by forced oscillation technique. (d-g) The number of the total, eosinophils, neutrophils, and lymphocytes in BALF were detected by trypan blue. All experiments were conducted in triplicate (** $P < 0.01$, *** $P < 0.001$, vs. Control; && $P < 0.01$, &&& $P < 0.001$, vs. OVA; # $P < 0.05$, ## $P < 0.01$, vs. OVA+Tio). (BLA: Bulleyaconitine A, AHR: Airway Hyperresponsiveness, OVA: ovalbumin, BALF: bronchoalveolar lavage fluid, Tio: tiotropium bromide).

with 50 µL enzyme conjugation liquid and incubated for 1 h at 37°C. After washing the well for 5 times with washing liquid, substrate A (50 µL) and B (50 µL) were added into each well, and incubated for 20 min at room temperature. Finally, the wells were added with 50 µL stopping liquid, and the absorbance was measured at 450 nm by a microplate reader (Infinite M200 PRO, Tecan Austria GmbH, Austria).

Statistical analysis

Student's t-test and one-way ANOVA were used to analyze the data by SPSS software (version 18.0). LSD

and Dunnett's were used as posthoc tests. The data were shown as mean ± standard. All experiments were conducted in triplicate. Statistically, a significant result was defined as $P < 0.05$.

Results

BLA attenuates AHR in chronic OVA-induced asthma and inhibits inflammatory cell recruitment in BALF

As AHR is the most typical characteristic of asthma [3], AHR of the mice in each group was detected. Experimental layout to develop the model of chronic

asthma in mice shown in Figure 1(a). As shown in Figure 1(b,c), the baselines of conditions of lung and airway resistances of these four groups of mice were almost the same, and with the increases of Mch at the concentration from 25 to 100 mg/mL (OVA group), the lung and airway resistances were significantly increased compared with the control groups ($P < 0.001$, respectively). However, in OVA+Tio and OVA+BLA groups, the treatment of Tio and BLA both inhibited the OVA-induced lung and airway resistances compared with OVA group ($P < 0.01$, respectively). Tio is a positive drug for asthma, and OVA+Tio group was seen as a positive control. The result revealed that BLA attenuated AHR in the mice with chronic OVA-induced asthma. Then, the change of total and differential leukocytes (eosinophils, neutrophils, and lymphocytes) in the BALF were detected, as shown in Figure 1(d,g), the total and differential leukocytes in OVA group were higher than those in control group ($P < 0.01$, respectively), while in OVA+Tio and OVA+BLA groups, the total and differential leukocytes were significantly reduced compared with OVA group ($P < 0.01$, respectively). Although the levels of eosinophils, neutrophils, and lymphocytes in OVA+BLA group were higher than that in OVA+Tio group ($P < 0.05$, respectively), the results still demonstrated that BLA inhibited inflammatory cell recruitment in BALF.

BLA inhibits the lung inflammation and IgE secretion in chronic OVA-induced asthma

Based on the current results, we further observed the changes in lung inflammation using H&E staining, as shown in Figure 2(a), histopathological examination showed that inflammation in perivascular and peribronchial regions was significantly increased in OVA group but reduced in OVA+Tio and OVA+BLA groups, however, the inflammation was not quantified. Then we measured the secretion levels of total IgE and OVA-specific IgE of the mice serum of each group. As shown in Figure 2(b,c), the results revealed that the secretion levels of total IgE and OVA-specific IgE were significantly increased in OVA group compared with control group ($P < 0.001$, respectively), while in OVA+Tio and OVA+BLA groups, the levels of the two were greatly reduced compared with OVA group ($P < 0.001$, respectively). Their results further demonstrated that BLA inhibited lung inflammation and IgE secretion in chronic OVA-induced asthma.

BLA inhibits the airway remodeling in chronic OVA-induced asthma

The airway remodeling in the mice of each group was observed, as shown in Figure 3(a), the level of mucous

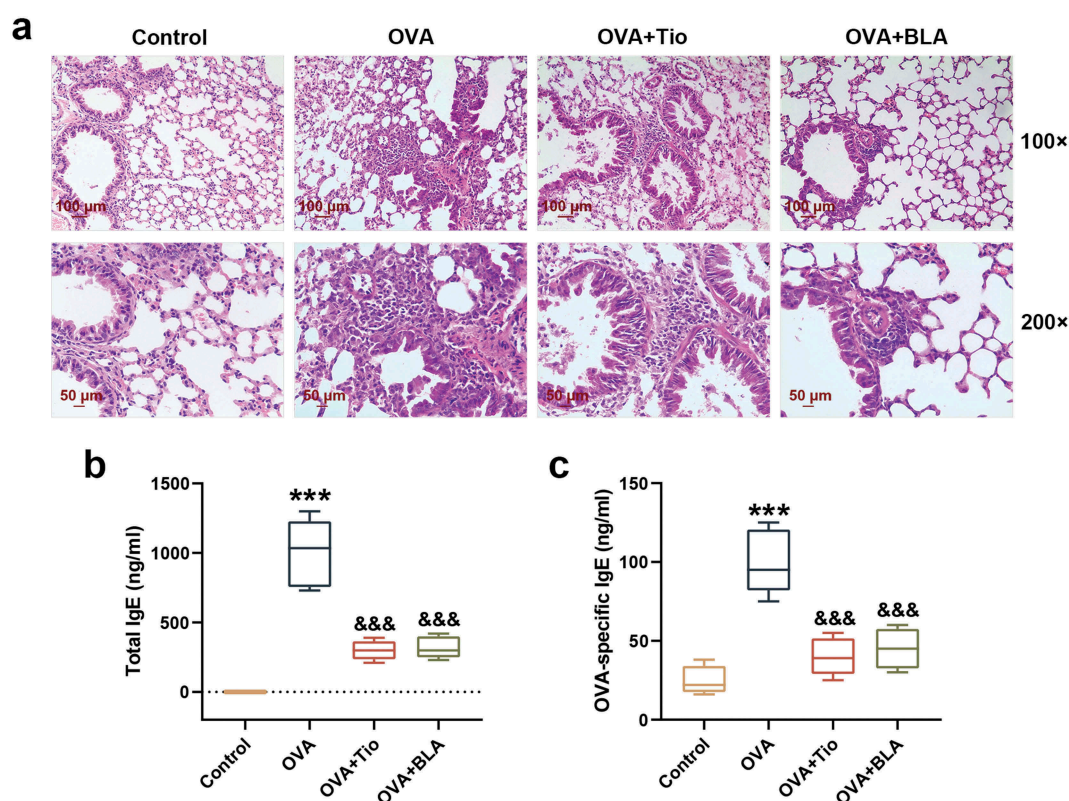


Figure 2. BLA inhibits the lung inflammation and IgE secretion in chronic OVA-induced asthma. (a). Lung histopathological examination was detected by H&E staining (Magnification $\times 100$, $\times 200$). (b, c) Total IgE and OVA-specific IgE secretion levels were detected by ELISA. All experiments were conducted in triplicate (*** $P < 0.001$, vs. Control; &&& $P < 0.001$, vs. OVA). (BLA: Bulleyaconitine A, OVA: ovalbumin, Tio: tiotropium bromide, H&E: hematoxylin and eosin, ELISA: enzyme-linked immunosorbent assay).

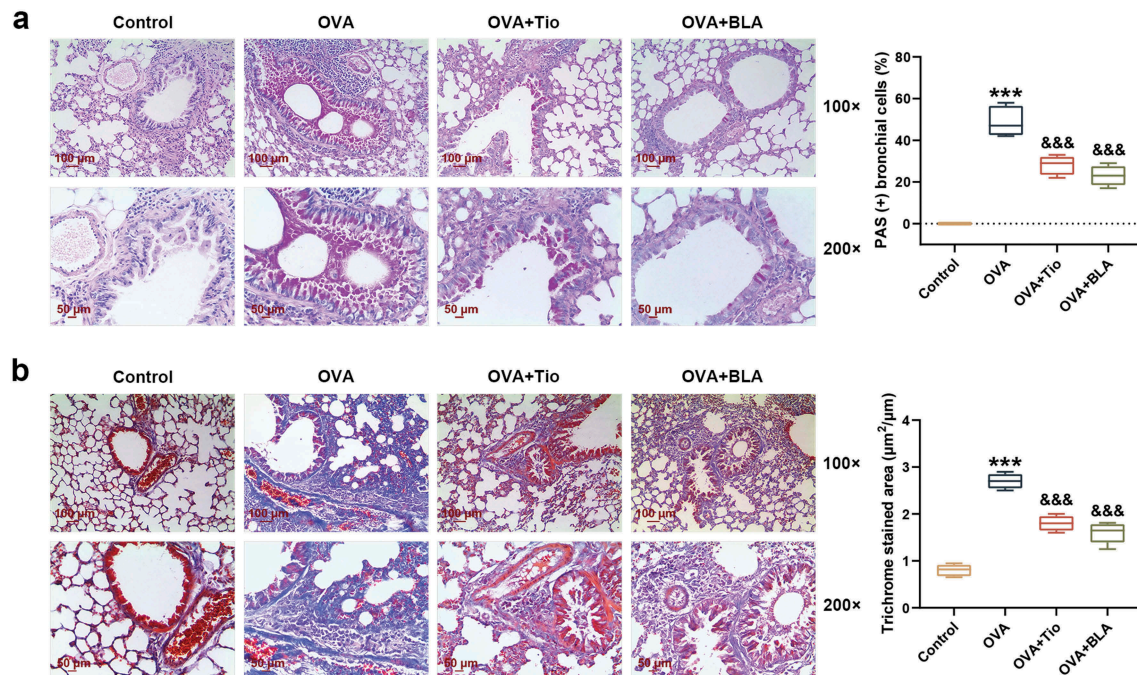


Figure 3. BLA inhibits the airway remodeling in chronic OVA-induced asthma. (a). The percentage of PAS+ epithelial cells out of the total number of epithelial cells in the airway in the lung tissues was detected by PAS staining. (b). The peribronchial area stained with Masson's trichrome was quantified to assess the extent of subepithelial fibrosis. All experiments were conducted in triplicate (** $P < 0.001$, vs. Control; &&& $P < 0.001$, vs. OVA). (BLA: Bulleyaconitine A, OVA: ovalbumin, Tio: tiotropium bromide, PAS: periodic acid-Schiff.).

secretion and the proportion of goblet cells (PAS⁺ cells) in OVA group were significantly increased compared with control group ($P < 0.001$), but noticeably reduced in OVA+Tio and OVA+BLA group compared with OVA group ($P < 0.001$, respectively). Similarly, in Figure 3(b), the area of peribronchial Masson staining (collagen deposition) in the OVA group was greatly promoted compared with control group ($P < 0.001$), but significantly reduced in OVA+Tio and OVA+BLA groups compared with OVA group ($P < 0.001$, respectively). The data further revealed that BLA could inhibit the airway remodeling in chronic OVA-induced asthma.

BLA inhibits the levels of Th2, Th17, and increases Th1 cytokines in BALF and serum

To determine the possible effect of BLA on T cell response in chronic OVA-induced asthma, the levels of Th1, Th2, and Th17 type cytokines in BALF and serum were detected. As shown in Figure 4, the levels of Th2-type cytokines (eotaxin, IL-4, IL-5, IL-13, and IL-33) and IL-17A were significantly increased in OVA group compared with the control group ($P < 0.001$), but noticeably reduced in OVA+Tio and OVA+BLA groups compared with OVA group ($P < 0.001$ or $P < 0.01$). In addition, the levels of Th1-type cytokines (IL-12 and IFN- γ) were significantly reduced in OVA group compared with control group ($P < 0.001$), but greatly increased in OVA+Tio and OVA+BLA groups compared with OVA group

($P < 0.01$). As shown in Figure 5, the secretion levels of these cytokines in the serums of the mice were consistent with that in BALF, indicating that the inhibitory effect of BLA on chronic OVA-induced asthma might be mediated through restoring Th1/Th2 cytokines balance.

Discussion

The current study aimed to explore the effects and mechanisms of BLA on asthma. After the model mice have been successfully established by OVA stimulation, the mice were treated by BLA. We found that BLA attenuated the OVA-induced lung and airway resistances, inhibited inflammatory cell recruitment in BALF, and also suppressed airway inflammation and airway remodeling, moreover, BLA inhibited the secretion of Th2-type and Th17 cytokines, but enhanced the secretion of Th1-type cytokines. These results revealed that BLA could suppress the AHR, inflammatory, and airway remodeling though restoring Th1/Th2 balance, indicating that BLA has the potential to be used in asthma treatment. Tio was used as a positive drug, and its efficacy on airway inflammation and programmed cell death 5 was evaluated in the model mice with ovalbumin-induced allergic asthma [17]. Ti attenuates virus-induced pulmonary inflammation in cigarette smoke-exposed mice [18]. Apart from functioning as a bronchodilator, Tio also affected airway inflammation. We found that Tio inhibited IgE production and Th2-type responses, and induced Th1-type responses.

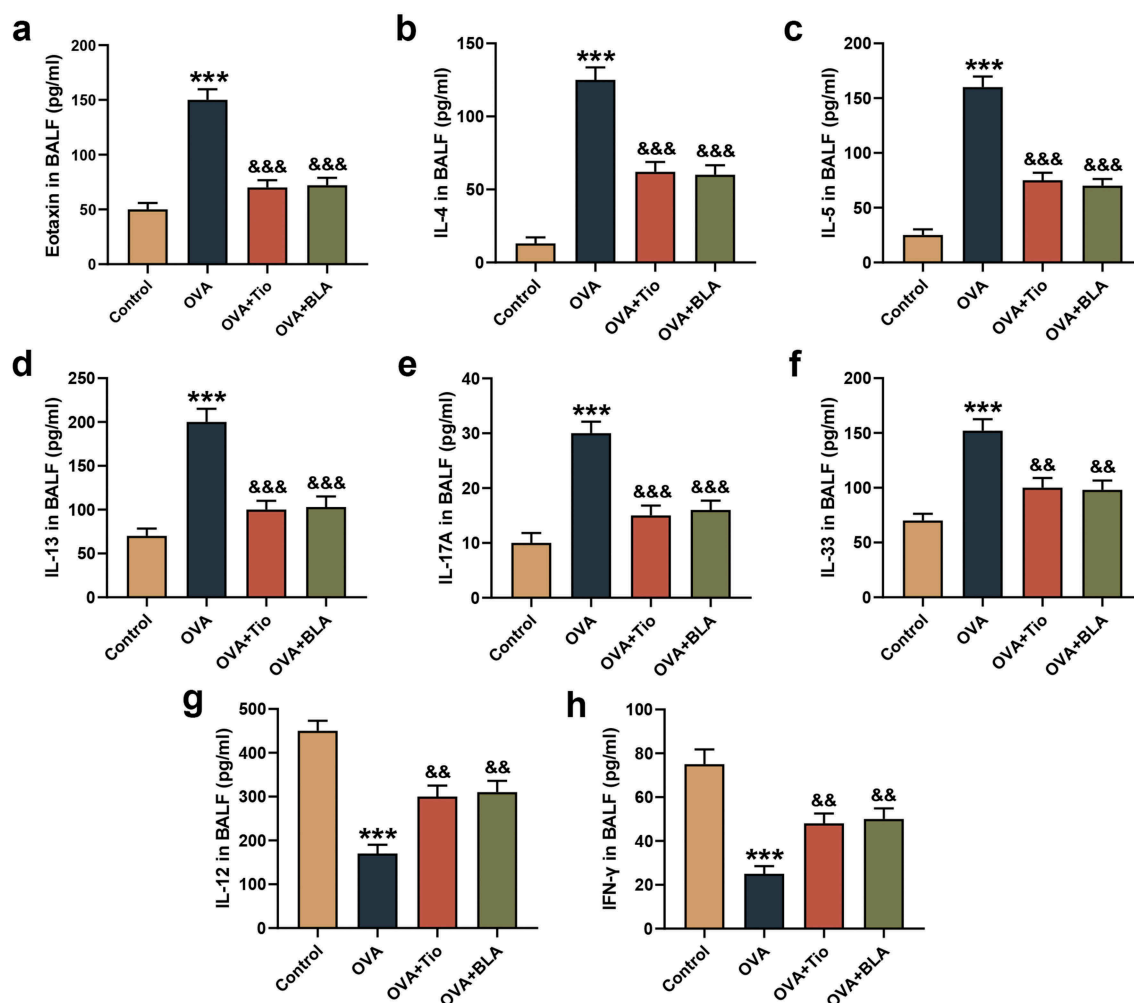


Figure 4. BLA inhibits the levels of Th2, Th17, and increases Th1 cytokines in BALF.

The secretion levels of Th2-type cytokines (eotaxin, IL-4, IL-5, IL-13, and IL-33), IL-17A, and Th1-type cytokines (IL-12 and IFN- γ) in mice BALF were detected by ELISA. All experiments were conducted in triplicate (** $P < 0.01$, vs. Control; && $P < 0.01$, &&& $P < 0.001$, vs. OVA). (BLA: Bulleyaconitine A, OVA: ovalbumin, Tio: tiotropium bromide, ELISA: enzyme-linked immunosorbent assay).

BLA has could enhance dynorphin secretion of the microglia and has an anti-hypersensitive effect [13]. In addition, research reported that BLA could inhibit lung inflammation in allergic asthma [14]. However, the effects of BLA on asthma and the underlying mechanism should be further explored and confirmed.

AHR is the final and a more direct symptom resulted from inflammation and airway remodeling [19]. Therefore, the changes in lung and airway resistances after BLA treatment were detected, and we found that lung and airway resistances of the asthma model mice stimulated by OVA were inhibited by BLA, indicating that BLA could inhibit AHR of the asthma mice. Airway inflammation plays an important role in the pathogenesis of asthma, and could lead to airflow obstruction [20]. Although it still remains controversial whether airway inflammation is the only cause of AHR, it is clear that inflammatory deposition is closely related to AHR and will lead to AHR [21]. In this study, we also found that BLA inhibited the recruitment of inflammatory cells and inflammation. In addition, chronic airway inflammation can further

contribute to the changes in airway structure leading to airway remodeling [22,23], the process of which consists of collagen deposition, sub-epithelial fibrosis, smooth muscle mass, wall thickening, goblet cell proliferation and angiogenesis [3,24]. Therefore, we further conducted related experiments, and further observed that BLA inhibited mucous secretion, reduced proportion of goblet cells, and collagen deposition in the lung tissues of asthma mice, suggesting that BLA also could inhibit the airway remodeling in the asthma mice. These results revealed that BLA could inhibit AHR, inflammatory cell recruitment, and airway remodeling.

Although inflammation plays an important role in the pathogenesis of asthma, its development is dependent on the release of various cytokines [25]. In addition, some clinical and genetic researches proved that allergen-specific Th2-type cytokines could drive the immunopathological characteristics of asthma [26]. Antigen-specific IgE is synthesized through some types of Th2 cytokines such as eotaxin, IL-4, IL-5, IL-13, and IL-33 [27]. For verifying whether BLA inhibited

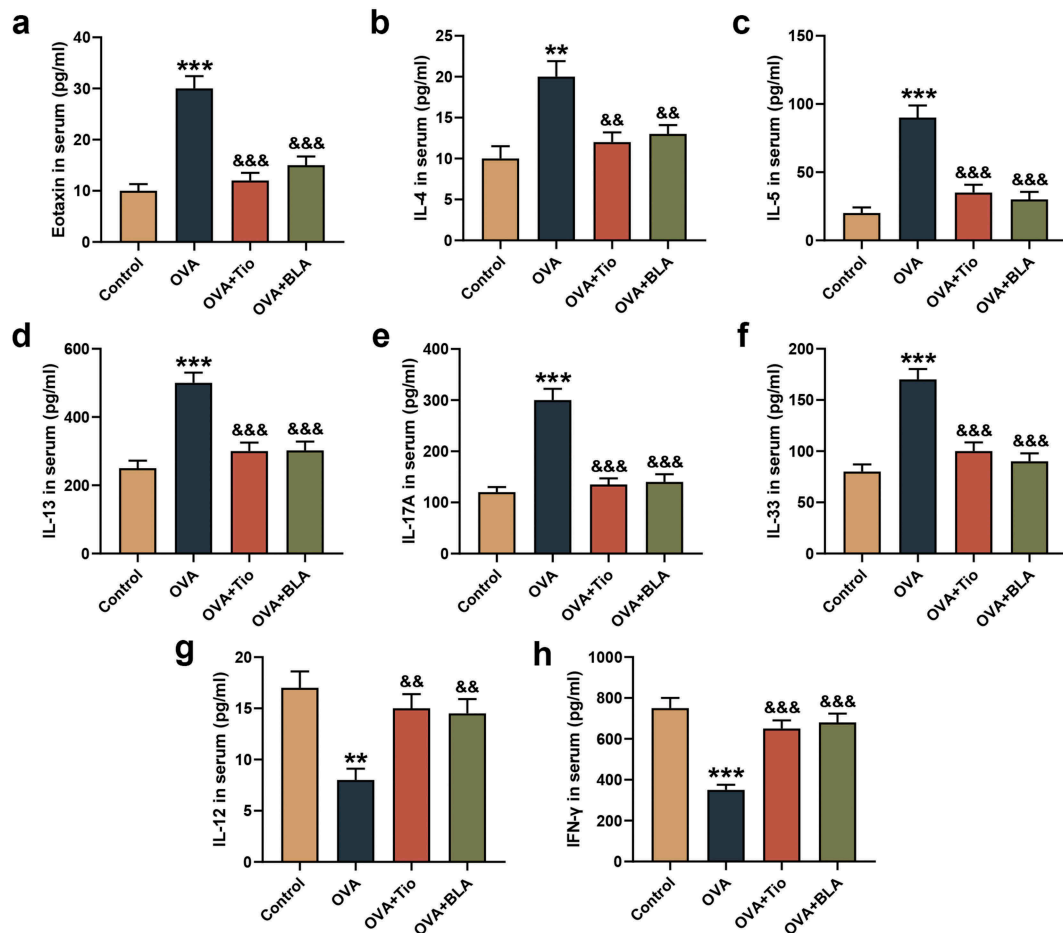


Figure 5. BLA inhibits the levels of Th2, Th17, and increases Th1 cytokines in serum.

The secretion levels of Th2-type cytokines (eotaxin, IL-4, IL-5, IL-13, and IL-33), IL-17A, and Th1-type cytokines (IL-12 and IFN- γ) in mice serum were detected by ELISA. All experiments were conducted in triplicate (** $P < 0.01$, *** $P < 0.001$, vs. Control; && $P < 0.01$, &&& $P < 0.001$, vs. OVA). (BLA: Bulleyaconitine A, OVA: ovalbumin, Tio: tiotropium bromide, ELISA: enzyme-linked immunosorbent assay).

the IgE level occurred before antigen inhalation or by the antigen inhalation, it is a limitation not detecting the IgE levels at day 14. Moreover, Th2-type cytokines, especially on eosinophils, also have a direct effect on the airway by chemotaxis of inflammatory cells to the inflammatory region [22,27,28]. Researches found that the activation of Th2 cytokines was associated with the severity of airway stenosis and AHR [20,29]. In addition, with the increased Th2 response, the level of IL-17, which belongs to Th17 cytokines, will be increased under asthmatic condition [30,31]. Therefore, in order to further confirm the current findings, we further detected the levels of cytokines of Th2 and Th17. Consistent with previous reports, we also found that Th2 and Th17 type of cytokines were high-expressed in OVA-induced mice, while BLA inhibited the secretion of these cytokines. It has been previously reported that the imbalance of Th1/Th2 cytokines is the main cause of asthma [32,33]. Therefore, Th1 cytokines also play important roles in the pathogenesis of asthma. Mice lack the cytokines of IFN- γ and IL-12, which belongs to Th1 cytokines, will exhibit the characteristics of allergic airway diseases [34]. Similarly, our result also

confirmed that Th1 type of cytokines was low-expressed in OVA-induced mice, while BLA promoted the secretion of these cytokines. Based on the current results, we concluded that BLA could restore Th1/Th2 balance in the model mice with asthma. At the same time, possible mechanisms, such as BLA blocking TTX-sensitive sodium channels, especially Nav1, also could be the mechanism underlying the anti-asthmatic effects of BLA. Moreover, there were possible relationship between inflammatory cell recruitment and changes in Th1 and Th2 cytokines.

In conclusion, the current study discovered that BLA inhibited AHR, lung inflammation, and airway remodeling via restoring Th1/Th2 balance in the murine asthmatic model. Whether BLA functions as a painkiller is dependent on or related to its anti-inflammatory effect should be evaluated in the future. Moreover, it would be interesting to continue the investigation on whether BLA shares a mutual mechanism with Tio in treating asthma, or BLA has anti-pain or bronchodilation activity. In addition, it would be inspiring to further study the effect of BLA on asthmatic patients.

Authors' contributions

Substantial contributions to conception and design: LL, SW

Data acquisition, data analysis and interpretation: HX, YS, JD, NH

Drafting the article or critically revising it for important intellectual content: LL, SW

Final approval of the version to be published: All authors

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: All authors

Disclosure statement

The authors declare no conflicts of interest.

Ethics approval and consent to participate

All animal experiments were performed in accordance with the guidelines of the China Council on Animal Care and Use. This study was approved by the Committee of Experimental Animals of Yantai Yuhuangding Hospital (Z20190105032H).

References

- [1] Papi A, Brightling C, Pedersen SE, et al. Asthma. *Lancet*. 2018 Feb 24;391(10122):783–800. PubMed PMID: 29273246; eng.
- [2] Svenningsen S, Nair P. Asthma endotypes and an overview of targeted therapy for asthma. *Front Med (Lausanne)*. 2017;4:158. PubMed PMID: 29018800; PubMed Central PMCID: PMC5622943.
- [3] Sethi GS, Sharma S, Naura AS. PARP inhibition by olaparib alleviates chronic asthma-associated remodeling features via modulating inflammasome signaling in mice. *IUBMB Life*. 2019 Jul;71(7):1003–1013. PubMed PMID: 30964965.
- [4] Cui J, Xu F, Tang Z, et al. Bu-Shen-Yi-Qi formula ameliorates airway remodeling in murine chronic asthma by modulating airway inflammation and oxidative stress in the lung. *Biomed Pharmacother*. 2019 Apr;112:108694. PubMed PMID: 30798140.
- [5] Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity*. 2019 Apr 16;50(4):975–991. PubMed PMID: 30995510; eng.
- [6] Fehrenbach H, Wagner C, Wegmann M. Airway remodeling in asthma: what really matters. *Cell Tissue Res*. 2017 Mar;367(3):551–569. PubMed PMID: 28190087; PubMed Central PMCID: PMC5320023. eng.
- [7] Quirt J, Hildebrand KJ, Mazza J, et al. Asthma. *Allergy Asthma Clin Immunol*. 2018;14(Suppl 2):50. PubMed PMID: 30275843; PubMed Central PMCID: PMC56157154. eng.
- [8] Loureiro CC, Amaral L, Ferreira JA, et al. Omalizumab for severe asthma: beyond allergic asthma. *Biomed Res Int*. 2018;2018:3254094. PubMed PMID: 30310816; PubMed Central PMCID: PMC56166383.
- [9] Zhu HQ, Xu J, Shen KF, et al. Bulleyaconitine A depresses neuropathic pain and potentiation at C-fiber synapses in spinal dorsal horn induced by paclitaxel in rats. *Exp Neurol*. 2015 Nov;273:263–272. PubMed PMID: 26376216; eng.
- [10] Xie MX, Zhu HQ, Pang RP, et al. Mechanisms for therapeutic effect of bulleyaconitine A on chronic pain. *Mol Pain*. 2018 Jan-Dec;14:1744806918797243. PubMed PMID: 30180777; PubMed Central PMCID: PMC56125851.
- [11] Xie MX, Pang RP, Yang J, et al. Bulleyaconitine A preferably reduces tetrodotoxin-sensitive sodium current in uninjured dorsal root ganglion neurons of neuropathic rats probably via inhibition of protein kinase C. *Pain*. 2017 Nov;158(11):2169–2180. PubMed PMID: 28915149.
- [12] Li TF, Wu HY, Wang YR, et al. Molecular signaling underlying bulleyaconitine A (BAA)-induced microglial expression of prodynorphin. *Sci Rep*. 2017 Mar 22;7:45056. PubMed PMID: 28327597; PubMed Central PMCID: PMC5361206.
- [13] Li TF, Fan H, Wang YX. Aconitum-derived bulleyaconitine A exhibits antihypersensitivity through direct stimulating dynorphin expression in spinal microglia. *J Pain*. 2016 May;17(5):530–548. PubMed PMID: 26762790; eng.
- [14] Zhan X, Zhang W, Sun T, et al. Bulleyaconitine A effectively relieves allergic lung inflammation in a murine asthmatic model. *Med Sci Monit*. 2019 Mar 4;25:1656–1662. PubMed PMID: 30828084; PubMed Central PMCID: PMC56413559.
- [15] Huang Z, Zhou X, Zhang J, et al. Bulleyaconitine A inhibits itch and itch sensitization induced by histamine and chloroquine. *Neuroscience*. 2020 Mar 1;429:68–77. PubMed PMID: 31935491; eng.
- [16] Xie MX, Yang J, Pang RP, et al. Bulleyaconitine A attenuates hyperexcitability of dorsal root ganglion neurons induced by spared nerve injury: the role of preferably blocking Nav1.7 and Nav1.3 channels. *Mol Pain*. 2018 Jan-Dec;14:1744806918778491. PubMed PMID: 29783906; PubMed Central PMCID: PMC565967161. eng.
- [17] Wang J, Diao X, Zhu H, et al. Effect of tiotropium bromide on airway inflammation and programmed cell death 5 in a mouse model of ovalbumin-induced allergic asthma. *Can Respir J*. 2019;2019:6462171. PubMed PMID: 31662808; PubMed Central PMCID: PMC56791200. eng.
- [18] Toumpanakis D, Loverdos K, Tzouda V, et al. Tiotropium bromide exerts anti-inflammatory effects during resistive breathing, an experimental model of severe airway obstruction. *Int J Chron Obstruct Pulmon Dis*. 2017;12:2207–2220. PubMed PMID: 28814849; PubMed Central PMCID: PMC565546183. eng.
- [19] Gon Y, Hashimoto S. Role of airway epithelial barrier dysfunction in pathogenesis of asthma. *Allergol Int*. 2018 Jan;67(1):12–17. PubMed PMID: 28941636.
- [20] Fahy JV. Type 2 inflammation in asthma—present in most, absent in many. *Nat Rev Immunol*. 2015 Jan;15(1):57–65. PubMed PMID: 25534623; PubMed Central PMCID: PMC4390063. eng.
- [21] Ano S, Panariti A, Allard B, et al. Inflammation and airway hyperresponsiveness after chlorine exposure are prolonged by Nrf2 deficiency in mice. *Free Radic Biol Med*. 2017 Jan;102:1–15. PubMed PMID: 27847240.
- [22] Amin K. The role of the T lymphocytes and remodeling in asthma. *Inflammation*. 2016 Aug;39(4):1475–1482. PubMed PMID: 27221139.
- [23] Kawayama T, Kinoshita T, Matsunaga K, et al. Role of regulatory T cells in airway inflammation in asthma. *Kurume Med J*. 2018 Apr 27;64(3):45–55. PubMed PMID: 29553094.

- [24] Boulet LP. Airway remodeling in asthma: update on mechanisms and therapeutic approaches. *Curr Opin Pulm Med.* **2018** Jan;24(1):56–62. PubMed PMID: 29076828; eng.
- [25] Zhao ST, Wang CZ. Regulatory T cells and asthma. *J Zhejiang Univ Sci B.* **2018** Sept;19(9):663–673. PubMed PMID: 30178633; PubMed Central PMCID: PMC6137416.
- [26] Robinson DS. The role of the T cell in asthma. *J Allergy Clin Immunol.* **2010** Dec;126(6):1081–91; quiz 1092–3. PubMed PMID: 20709383.
- [27] Deo SS, Mistry KJ, Kakade AM, et al. Role played by Th2 type cytokines in IgE mediated allergy and asthma. *Lung India.* **2010** Apr;27(2):66–71. PubMed PMID: 20616938; PubMed Central PMCID: PMC612893428.
- [28] Nakagome K, Nagata M. Involvement and possible role of eosinophils in asthma exacerbation. *Front Immunol.* **2018**;9:2220. PubMed PMID: 30323811; PubMed Central PMCID: PMC6172316.
- [29] Kay AB. TH2-type cytokines in asthma. *Ann N Y Acad Sci.* **1996** Oct 31;796:1–8.
- [30] Shi YH, Shi GC, Wan HY, et al. Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with allergic asthma. *Chin Med J (Engl).* **2011** Jul 5;124(13):1951–1956. PubMed PMID: 22088452.
- [31] Chesne J, Braza F, Mahay G, et al. IL-17 in severe asthma. Where do we stand? *Am J Respir Crit Care Med.* **2014** Nov 15;190(10):1094–1101. PubMed PMID: 25162311; eng.
- [32] Woodfolk JA. Cytokines as a therapeutic target for allergic diseases: a complex picture. *Curr Pharm Des.* **2006**;12(19):2349–2363. PubMed PMID: 16842183; eng.
- [33] Diao M, Min J, Guo F, et al. Effects of salbutamol aerosol combined with magnesium sulfate on T-lymphocyte subgroup and Th1/Th2 cytokines of pediatric asthma. *Exp Ther Med.* **2017** Jan;13(1):117–120. PubMed PMID: 28123478; PubMed Central PMCID: PMC615245138.
- [34] Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol.* **2003** Mar;111(3):450–63; quiz 464. PubMed PMID: 12642820; eng.