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Tiarellic acid attenuates airway hyperresponsiveness and inflammation in a murine model of allergic asthma

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ABSTRACT

Asthma is a persistent inflammatory disease characterized by airway obstruction and hyperresponsiveness in association with airway inflammation. In the current research, we studied the anti-inflammatory and anti-asthmatic effects of tiarellic acid (TA) isolated from *Tiarella polyphylla*, based on asthmatic parameters, such as immunoglobulin E (IgE) level, cytokine release, eosinophilia, airway hyperresponsiveness (AHR), reactive oxygen species (ROS) and mucus hypersecretion, in an ovalbumin (OVA)-sensitized/challenged mouse model. TA significantly inhibited increases in IgE, levels of ROS and T helper cytokines, such as interleukin (IL)-4, IL-5, TNF- α , and IL-13, in bronchoalveolar lavage fluid (BALF), and effectively suppressed airway hyperresponsiveness, eosinophilia, and mucus hypersecretion in the asthmatic mouse model. In addition, we found that administration of TA attenuated ovalbumin-induced increases in NF- κ B activity in lungs. The efficacy of TA was comparable to that of montelukast, a currently available anti-asthmatic drug. Our results support the utility of TA as a herbal medicine for asthma treatment and may have application in the development of anti-inflammatory and anti-asthmatic drugs.

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1. Introduction

Asthma is a chronic respiratory disease of the airway characterized by aggravation of inflammation, airway hyperresponsiveness (AHR) and oxidative stress [1]. The pathogenesis of asthma is associated with infiltration of inflammatory cells, including eosinophils, mast cells, macrophages, and T helper (T_H) cells [2]. Data obtained using animal models of asthma have suggested that T helper type 2 (Th2) lymphocytes are induced via production of cytokines through an inflammatory cascade comprising eosinophil action, immunoglobulin (IgE) production, mast cell activation, and reactive oxygen species (ROS) production, all of which produce the necessary mediators causing AHR [3–6].

Th2 cytokines, including interleukin (IL)-4, IL-5, and IL-13, are essential for generating the pathophysiological features of asthma. IL-4 and IL-13 play important roles in immunoglobulin (Ig)E switching in B cells, mucus hypersecretion, and eosinophil infiltration into lung tissue [7]. Eosinophils are additionally activated by IL-5, which induces the release of pro-inflammatory mediators and ROS [8]. Moreover, ROS play an important role in allergic disorders, and increased levels of oxidants are markers of the inflammatory process. Overproduction of ROS induces oxidative stress in the airway and lung tissue, leading to

progression of inflammation in the asthmatic airway [9,10]. Accordingly, many researchers have focused on modulation of Th2 cytokines or reduction of ROS for the treatment of allergic asthma [10,11].

Tiarellic acid (TA) is a natural product extracted from *Tiarella polyphylla* D. Don, a herb belonging to the Saxifragaceae family. *T. polyphylla* is distributed in China, Japan, Taiwan and Korea [12], and is specifically grown on the Ulleung island of Korea [13]. The whole *T. polyphylla* plant is traditionally used for the treatment of bruises and hearing difficulties. The main active components of *T. polyphylla* are some triterpenoids and seven oleanolic saponins [14]. Recently, oleanolic saponins have been isolated from plants and their anti-complementary activities tested, with the lupin-type triterpene, tiarellic acid, showing a strong apoptosis induction effect [15,16]. However, to date, no studies have focused on the anti-asthmatic properties of TA. In this study, we investigated the protective effects of TA on asthmatic responses using an ovalbumin (OVA)-induced allergic asthma model.

2. Materials and methods

2.1. Extraction and purification of TA

Dried whole *T. polyphylla* (30.7 kg) plants were extracted with MeOH (100 l each time) for a week at room temperature. The procedure was repeated 3 times. The combined extract solution was concentrated using a vacuum evaporator, providing 3.5 kg of residue. The MeOH

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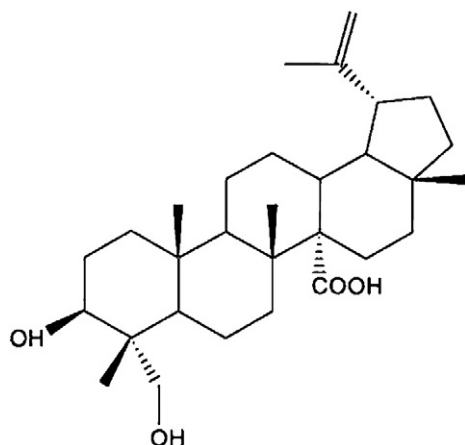


Fig. 1. Chemical structure of TA.

extract was suspended in H₂O and successively partitioned with hexane and EtOAc, leaving the residual water-soluble fraction. The hexane-soluble fraction (565.0 g) was subjected to chromatography on a silica gel column (18×50 cm) and the gradient eluted with CHCl₃/MeOH (100:0 to 0:100) to yield eight fractions (TPH-1 to TPH-8). Structure of TA was shown in Fig. 1, TA (3.9 g) obtained from fraction TPH-6 (74.0 g) was applied to a silica gel column (10×60 cm), and the gradient eluted with CHCl₃/MeOH (100:0 to 0:100) [17]. HPLC (MeOH 58% + 0.2% TFA 42%) of TA (0.1 µg/µl, 3,23-dihydroxy-20(29)-lupen-27-oic acid, [28]) was performed using Finnigan Surveyor HPLC system with column of Perfectsil Target ODS-3.

2.2. Animals

Pathogen-free female BALB/c mice (6–8 weeks old) were obtained from Orient Co. (Seoul, Korea). Mice were maintained in an animal facility under standard laboratory conditions for 1 week prior to experiments and provided water and standard chow *ad libitum*. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and animal handling followed the dictates of the National Animal Welfare Law of Korea.

2.3. Sensitization and airway challenge

Mice were divided into four groups ($n=6$ or 7), and airway inflammation induced with ovalbumin (OVA, grade III; Sigma-Aldrich, USA) in three of the groups using the method described by Oh et al. [18]. Mice were immunized *via* intraperitoneal (i.p.) injection with 20 µg chicken OVA and 2 mg aluminum hydroxide in 200 µL PBS (pH 7.4) on days 0 and 14. Subsequently, mice were exposed to 1% (w/v) OVA solution in PBS for 20 min using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan) on days 28, 29, and 30 after the initial sensitization. Animals were sacrificed 48 h after the final

challenge (day 32) to characterize the suppressive effects of TA. A schematic diagram of the treatment schedule is shown in Fig. 2.

Montelukast is a cysteinyl leukotriene (cys-LT)-1 receptor antagonist [19], and useful clinical responses to this agent have been obtained in patients with aspirin-sensitive asthma, nocturnal exacerbation of asthma, and allergic asthma, leading to successful commercialization of the drug [20]. In the present study, montelukast was used as a positive control.

2.4. Measurement of airway hyperresponsiveness (AHR)

At 24 h after the final aerosol challenge, AHR was assessed in conscious, unrestrained mice by means of whole-body plethysmography (OCP3000; Allmedicus, Seoul, Korea). Each mouse was placed in a plastic chamber and exposed to aerosolized PBS, followed by delivery of aerosols containing increasing concentrations of methacholine (6.25, 12.5, and 25 mg/mL; Sigma, St Louis, MO) in PBS for 3 min at individual exposure levels. Bronchoconstriction was recorded for an additional 5 min after each methacholine dose. The highest Penh value obtained during each methacholine challenge was expressed as a proportion of the basal Penh value recorded in response to PBS aerosol.

2.5. Inflammatory cell counts in bronchoalveolar lavage fluid (BALF)

Mice were sacrificed using an overdose of 50 mg/kg of pentobarbital 48 h after the last challenge, and tracheotomy performed. After instilling ice-cold PBS (0.5 mL) into the lung, BALF was obtained by three successive aspirations (total volume, 1.5 mL) *via* tracheal cannulation [21]. BALF was centrifuged (250 g, 4 °C, for 5 min) and each supernatant fraction collected and stored at −70 °C. Cell pellets were assessed by counting of cells in at least five squares of a hemocytometer after exclusion of dead cells stained with trypan blue [10]. Aliquots of BALF (100 µL) were placed on a slide and centrifuged (200 g, 10 min, at 4 °C) to fix cells using a cytospin machine (Hanil Science Industrial, Seoul, Korea). After slides were dried, cells were fixed and stained using Diff-Quik® Stain reagents (B4132-1A; Dade Behring Inc., Deerfield, IL), according to the manufacturer's instructions.

2.6. 2',7'-Dichlorofluorescein (DCF) fluorescence assay

Induction of oxidative stress was monitored using 2',7'-dichlorofluorescein (DCF-DA, Molecular Probes, Eugene, OR), which is converted to the highly fluorescent DCF by cellular peroxides, including hydrogen peroxide. In brief, cells from BALF were washed with PBS and total cells were counted and next treated with 25 µM DCF-DA for 10 min at 37 °C. To measure intracellular ROS activity, fluorescence was determined at 488 nm excitation and 525 nm emission on a fluorescence plate reader (Perkin-Elmer, Waltham, MA).

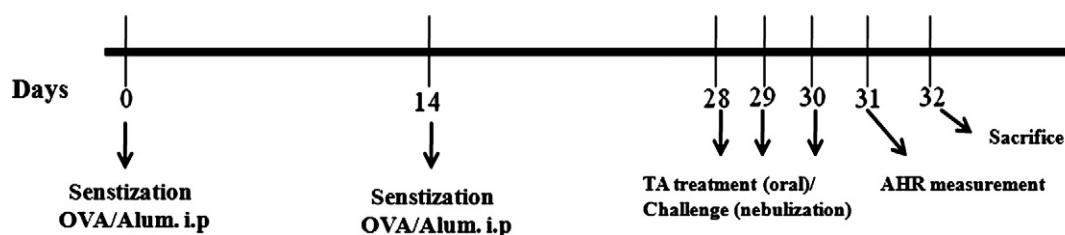


Fig. 2. Experimental protocol for induction of airway inflammation and treatment with TA using a mouse model. Mice were divided into four groups (6–7 animals/group) and sensitized *via* intraperitoneal injection of 20 µg OVA emulsified in 2 mg aluminum hydroxide in 200 µL PBS buffer (pH 7.4) on days 0 and 14. Individual mice were challenged through the airway with OVA (1%, w/v, in PBS) for 20 min using an ultrasonic nebulizer on days 28, 29, and 30 after initial sensitization. Mice were administered 10 or 30 mg/kg/day of TA orally on days 28 to 30. Animals were sacrificed 48 h after the final challenge.

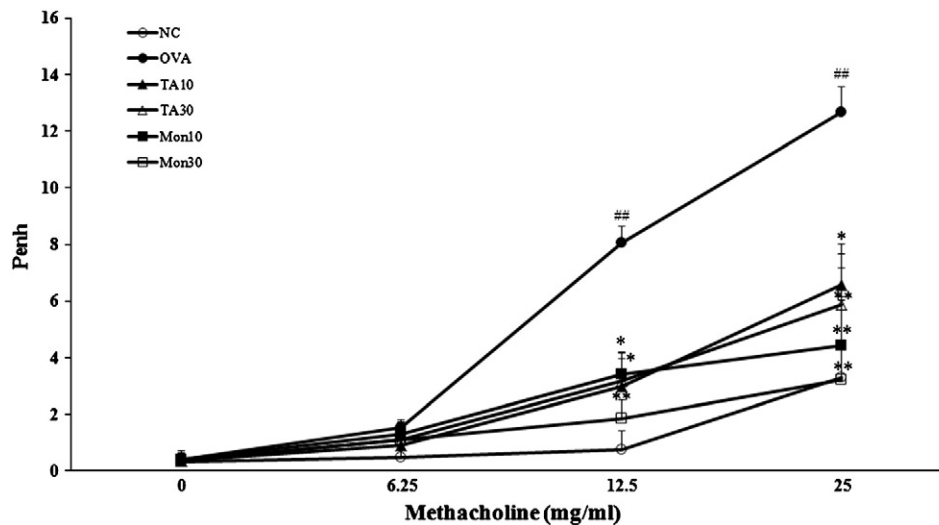


Fig. 3. Effect of TA on airway hyperresponsiveness. AHR was measured 24 h after the final OVA challenge in mice exposed to increasing doses of methacholine (6.25–25 mg/mL). NC, normal control (PBS only); OVA, OVA-induced mice; TA10 and 30, tiarellic acid (10, 30 mg/kg) + OVA-induced mice; Mon10 and 30, Montelukast (10, 30 mg/kg) + OVA-induced mice. Values are expressed as means $\pm$ SD. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, significantly different from NC. $^*P < 0.05$ and $^{**}P < 0.01$, significantly different from OVA.

2.7. Measurement of total and OVA-specific IgE levels in BALF

The concentrations of total and OVA-specific-IgE in BALF and serum were measured using sandwich enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with 100 μ L/well IgE (2 μ g/mL; Serotec, Oxford, UK) in PBS. Antibodies were detected in BALF using isotype-specific secondary antibodies (anti-mouse IgE; Serotec). After four washes, 200 μ L of *o*-phenylenediamine dihydrochloride (Sigma, St Louis, MO) was added to each well. Plates were incubated for 10 min in the dark, and absorbance measured at 450 nm. Total and OVA-specific IgE concentrations were calculated from a standard curve generated using 250 ng/mL recombinant IgE (Serotec, Oxford, UK).

2.8. Measurement of cytokine levels in BALF

Enzyme-linked immunosorbent assays (ELISA) were performed according to the manufacturer's instructions. The concentrations

of IL-4, IL-5, TNF- α , and IL-13 in BALF were measured using specific mouse IL-4, IL-5 and IL-13 ELISA kits (Biosource, Carlsbad, CA).

2.9. Lung tissue histopathology

After BALF was obtained, lung tissue was fixed in 10% (v/v) neutral buffered formalin for 24 h. Tissues were subsequently embedded in paraffin, sectioned at 4 μ m thickness, and stained with H&E solution (hematoxylin; Sigma, MHS-16; and eosin; Sigma, HT1100-1-32) and periodic acid-Schiff (PAS) (IMEB Inc., San Marcos, CA) to estimate mucus production. Tissues were subsequently mounted and coverslipped using Dako mounting medium (Dakocytomation, Denmark, CA). Photomicrographs were obtained using a Photometric Quantix digital camera running a Windows program, and the montages assembled in Adobe Photoshop 7.0. Images were cropped

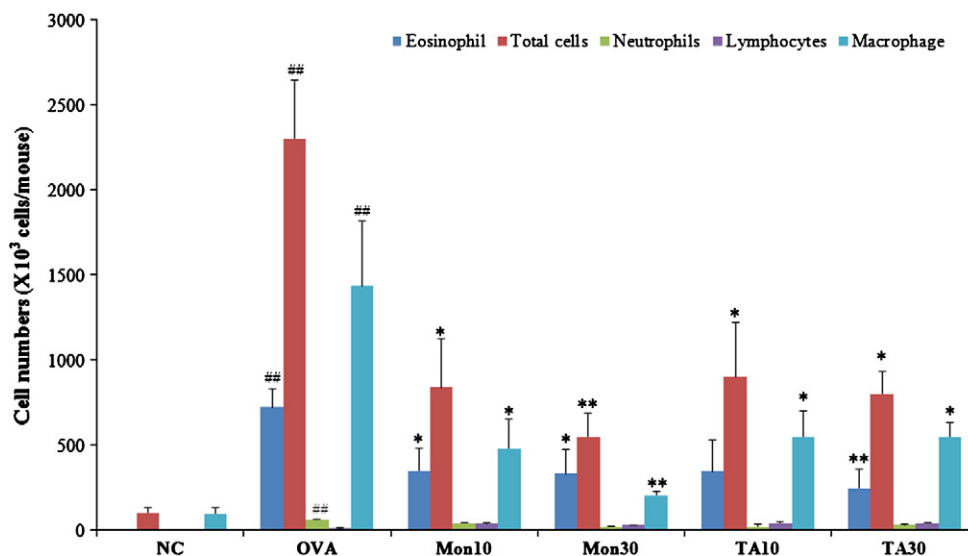


Fig. 4. Effect of TA on recruitment of inflammatory cells into BALF. Cell numbers was assessed by direct counting under a light microscope in at least five squares of a hemocytometer after exclusion of dead cells stained with trypan blue. NC, normal control (PBS only); OVA, OVA-induced mice; TA10 and 30, tiarellic acid (10, 30 mg/kg) + OVA-induced mice; Mon10 and 30, Montelukast (10, 30 mg/kg) + OVA-induced mice. Values are expressed as means $\pm$ SD. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, significantly different from NC. $^*P < 0.05$ and $^{**}P < 0.01$, significantly different from OVA.

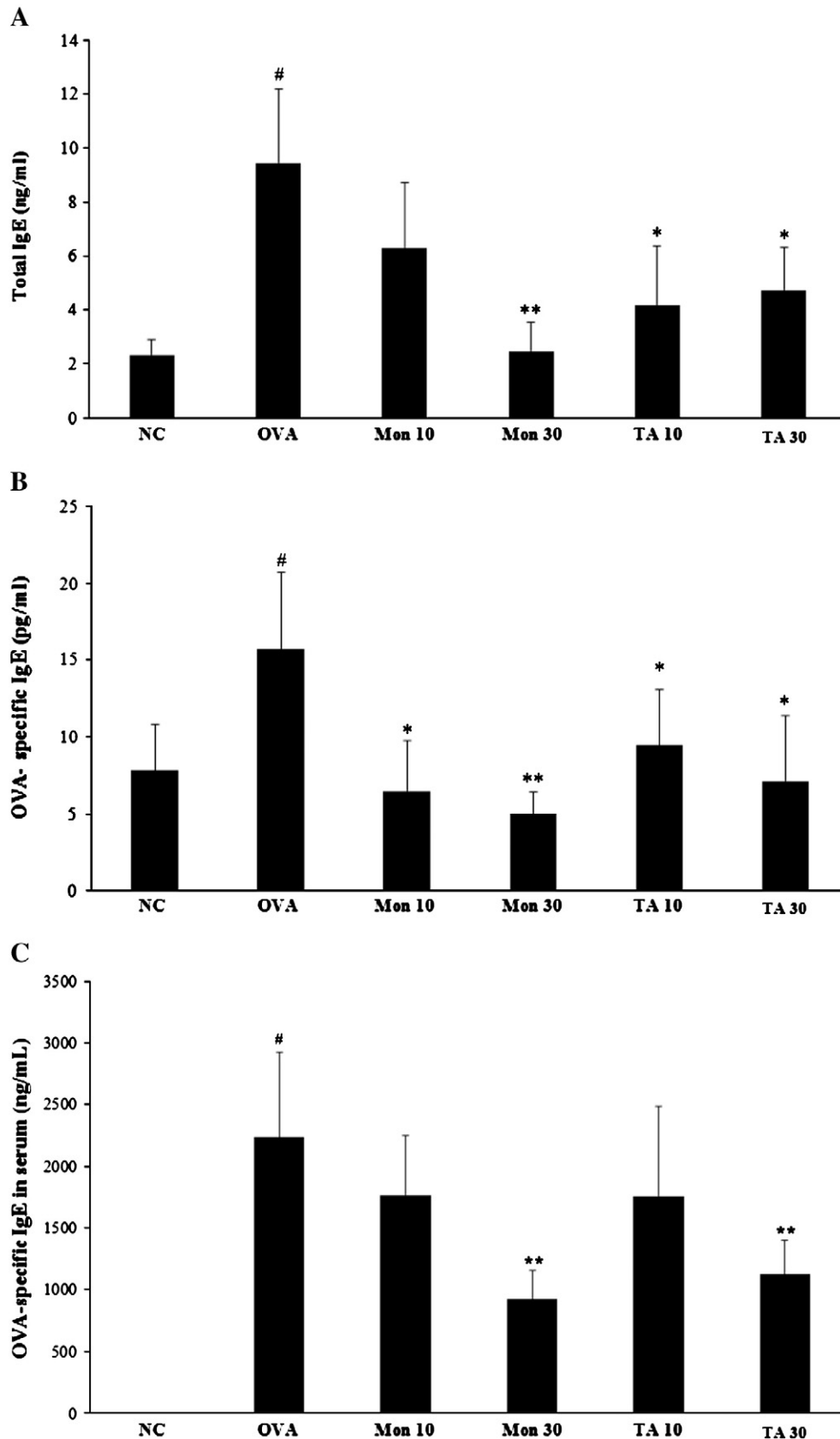


Fig. 5. Effects of TA on total and OVA-specific IgE levels in BALF. (A) Total IgE in BALF (B) OVA-specific IgE in BALF (C) OVA-specific IgE in serum were analyzed with ELISA ($n = 6$ or 7 animals). NC, normal control (PBS only); OVA, OVA-induced mice; TA10 and 30, tiarellic acid (10, 30 mg/kg) + OVA-induced mice; Mon10 and 30, Montelukast (10, 30 mg/kg) + OVA-induced mice. Values are expressed as means $\pm$ SD. # $P < 0.05$ and ## $P < 0.01$, significantly different from NC. * $P < 0.05$ and ** $P < 0.01$, significantly different from OVA.

Table 1
Effects of TA on cytokines in BLAF.

Groups	IL-4 (pg/mL)	IL-5 (pg/mL)	IL-13 (pg/mL)	TNF- α (pg/mL)
NC	62.3 $\pm$ 19.5	72.4 $\pm$ 13.1	42.8 $\pm$ 3.67	142.8 $\pm$ 21.3
OVA	115.4 [*] $\pm$ 15.2	101.3 [*] $\pm$ 5.7	113.4 [*] $\pm$ 13.42	218.4 [*] $\pm$ 15.8
Mon10	78.2 [†] $\pm$ 9.1	45.6 [†] $\pm$ 7.8	61.1 [†] $\pm$ 11.8	192.5 $\pm$ 13.2
Mon30	58.4 [†] $\pm$ 13.4	38.4 [†] $\pm$ 9.7	50.8 [†] $\pm$ 16.3	145.7 [†] $\pm$ 15.5
TA10	66.3 [†] $\pm$ 20.3	48.0 [†] $\pm$ 5.3	60.8 $\pm$ 7.8	194.6 $\pm$ 19.6
TA30	47.2 [†] $\pm$ 8.4	48.1 [†] $\pm$ 6.5	53.8 [†] $\pm$ 10.3	163.5 [†] $\pm$ 17.4

* Significantly different from NC, $P < 0.05$.

[†] Significantly different from OVA, $P < 0.05$.

and corrected for brightness and contrast, but not otherwise manipulated.

2.10. Western blot

Equal amounts of total lung protein (30 μ g) were heated at 100 $^{\circ}$ C for 5 min and then loaded onto 8% SDS-PAGE gels, followed by transfer to a nitrocellulose membrane (at 100 V for 2 h). The membranes were blocked for 1 h with Tris-buffered saline containing 0.05% Tween 20 (TBST) plus 5% skim milk and were incubated with anti-ICAM (1:1000 dilution), anti-VCAM (1:1000 dilution), anti-NF- κ B p65 and anti- β -actin (1:1000 dilution) overnight at 4 $^{\circ}$ C. The membranes were washed three times with TBST, and then incubated with a 1:10,000 dilution of horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. The membranes were again washed three times with TBST, and then developed using an enhanced chemiluminescence kit.

2.11. Statistical analysis

Data are expressed as means $\pm$ SD (standard deviations of the mean). Statistical comparisons were made with one-way analysis of variance (ANOVA) using SPSS 16.0, followed by Duncan's new multiple range test, using $P < 0.05$ as the level of significance.

3. Results

3.1. Effect of TA on AHR

AHR was evaluated by calculating Penh values (enhanced pauses) 24 h after the final OVA challenge. The Penh value of the OVA-induced group was significantly higher than that of the PBS control group ($P < 0.05$) when concentrations of 6.5–25 mg/mL methacholine were delivered in aerosols. OVA-induced mice treated with TA displayed reduced mean Penh values upon exposure to 25 mg/mL methacholine, compared with the response of the OVA-induced group ($P < 0.05$). Positive control mice administered montelukast showed a AHR decrease similar to that achieved using TA (Fig. 3).

3.2. Effect of TA on OVA-induced eosinophilia in BALF

We examined changes in total cells, eosinophils, neutrophils, lymphocytes, and macrophages in BALF to determine the effects of TA on asthma. As shown in Fig. 4, OVA induced a marked influx of leukocytes into BALF in the PBS control group. Eosinophils constituted less than 1% of the total cell content of PBS-treated mice. However, eosinophils were markedly increased to levels greater than those of total leukocytes in BALF of OVA-challenged mice. OVA-induced mice treated with TA displayed significantly reduced leukocytosis, decreased absolute eosinophilia, and decreased lymphocytosis upon asthma induction. The positive drug, montelukast, exerted similar suppressive effects on leukocyte influx into BALF (Fig. 4).

3.3. Effects of TA on the total and OVA-specific IgE levels in BALF and serum

We further examined whether TA influences total and OVA-specific IgE production in the allergic asthma mouse model, in view of the finding that antigen-specific Th2 responses trigger IgE antibody production. The concentrations of total and OVA-specific IgE in BALF were determined in each experimental group 48 h after the final OVA challenge using ELISA. Total and OVA-specific IgE levels in BALF and serum were markedly increased in OVA-treated mice, compared

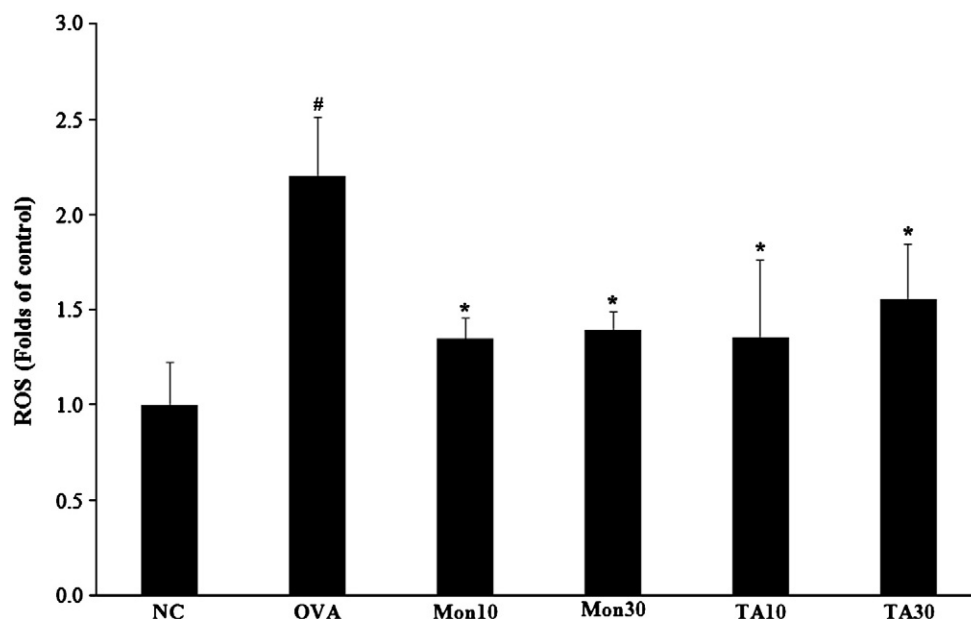


Fig. 6. Effect of TA on ROS generation in BALF. Oxidative stress was quantified in BALF, based on DCF fluorescence. Values represent relative fluorescence units per milligram protein. NC, normal control (PBS only); OVA, OVA-induced mice; TA10 and 30, tiarellic acid (10, 30 mg/kg) + OVA-induced mice; Mon10 and 30, Montelukast (10, 30 mg/kg) + OVA-induced mice (thick hatched bars). # $P < 0.05$ and ** $P < 0.01$, significantly different from NC. * $P < 0.05$ and ** $P < 0.01$, significantly different from OVA.

with PBS-treated mice. Notably, TA suppressed both total and OVA-specific IgE levels in BALF, compared to those in the OVA-induced group. The positive drug, montelukast, had a similar suppressive effect (Fig. 5).

3.4. Effects of TA on cytokine and chemokine levels in BALF

To establish the mechanisms underlying the inhibitory activity of TA in airway inflammation, we examined the Th2 cytokine levels associated with asthma in BALF. IL-4, IL-5, and IL-13 levels in BALF were markedly increased in OVA-induced mice, compared with those in PBS-treated mice. Administration of TA (30 mg/kg) led to a significant decrease in the levels of all Th2 cytokines examined (IL-4, IL-5 and IL-13) (Table 1) in BALF in relation to those in the OVA-induced group.

3.5. Effect of TA on OVA-induced ROS generation in BALF cells

As illustrated in Fig. 6, BALF cells of OVA-induced mice contained significantly increased ROS levels. However, pre-administration of TA led to a significant decrease in ROS generation in BALF cells.

3.6. Effect TA on OVA-induced eosinophilia in lung tissue

To further examine the anti-asthmatic and anti-remodeling effects of TA, lung tissues were collected 48 h after the final OVA challenge. In OVA-induced asthmatic lung, we observed marked infiltration of inflammatory cells into perivascular and peribronchial connective tissue, compared with the PBS control group. Moreover, the majority of leukocytes were eosinophils. The PBS control group displayed no change in the extent of inflammatory cell infiltration, similar to montelukast-treated positive control mice. Eosinophilia was significantly attenuated

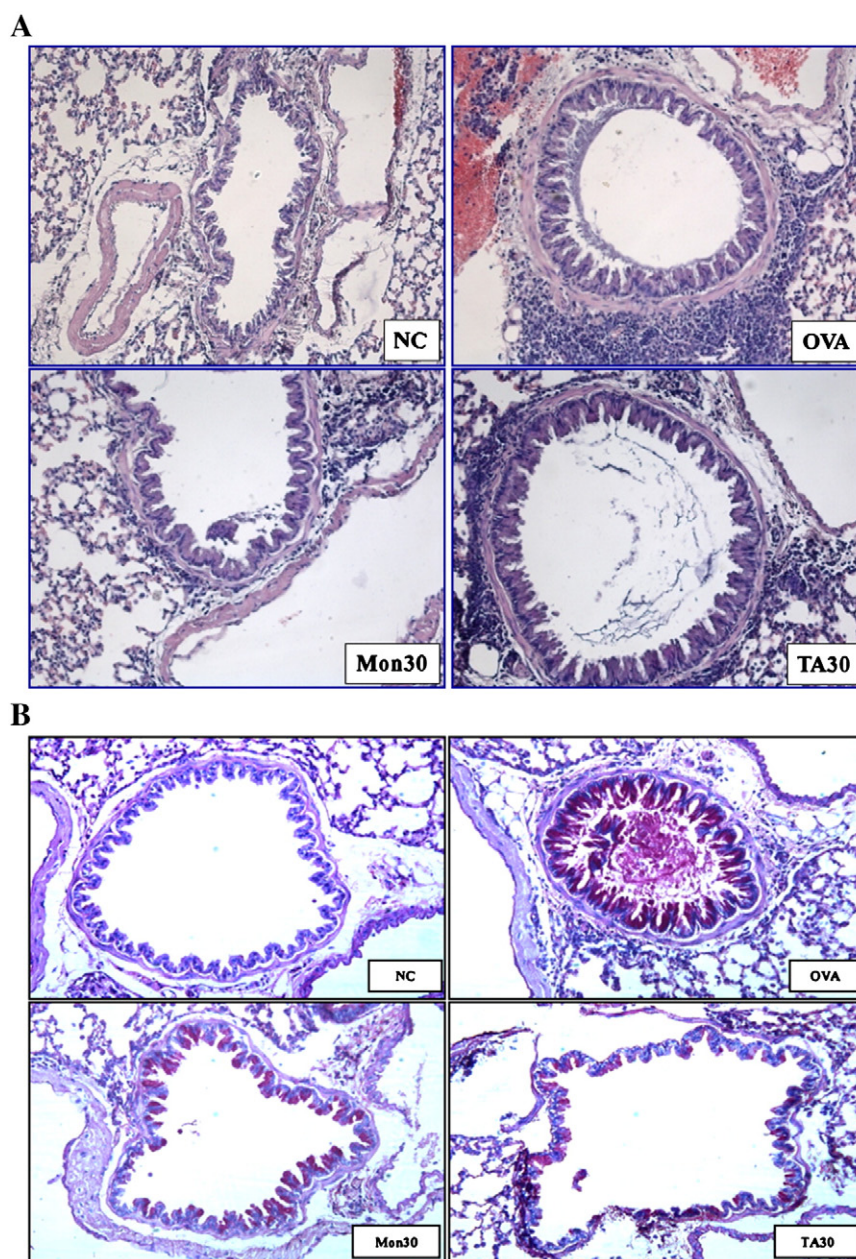


Fig. 7. Effect of TA on leukocyte recruitment and mucus production in lung tissue. Histological examination of lung tissue was performed 48 h after the final OVA challenge. Lung tissues were fixed, sectioned at 4 μ m thickness, and stained with (A) H&E and (B) periodic acid-Schiff (PAS) reagent (magnification $\times 200$). NC, normal control (PBS only); OVA, OVA-induced mice; TA30, tiarellic acid (30 mg/kg) + OVA-induced mice; Mon30, Montelukast (30 mg/kg) + OVA-induced mice.

Table 2

Scoring of the extent of inflammation via quantitative analysis of inflammatory cell infiltration in lung sections.

Groups	Mucus score
NC	<0
OVA	3.2* ± 0.71
Mon30	1.8 [†] ± 0.62
TA30	2.1 [†] ± 0.67

* Significantly different from NC, $P < 0.05$.

[†] Significantly different from OVA, $P < 0.05$.

in OVA-induced mice treated with TA, compared with that in OVA-induced mice (Fig. 7A).

3.7. Effects of TA on airway goblet cell hyperplasia and mucus production

Mucus hypersecretion is a characteristic of airway remodeling. The number of goblet cells containing mucus was increased in OVA-induced mice. TA attenuated OVA-induced infiltration of inflammatory cells. To determine whether TA suppresses mucus overproduction caused by goblet cell hyperplasia, we stained lung sections with PAS. Compared with PBS-treated animals, mucus overproduction was clearly observed as a violet color in bronchial airways in OVA-induced mice. In contrast, the extent of mucus staining was markedly diminished in OVA-induced mice treated with TA (Fig. 7B and Table 2). Our data confirm that TA significantly reduces goblet cell hyperplasia and mucus hypersecretion characteristic of the airway remodeling process.

3.8. Effect of astilbic acid on NF- κ B p65 protein levels in lung tissue

We assessed the effect of TA on NF- κ B p65 levels during OVA-induced allergic airway inflammation. OVA-sensitized and -challenged mice displayed increased concentrations of NF- κ B p65 in nuclear protein extracts from lung tissues, compared with the PBS control group (Fig. 8). Conversely, the NF- κ B p65 level was significantly lower in the TA-treated group.

4. Discussion

We evaluated the protective effects of TA against airway inflammation using a murine model of OVA-induced allergic asthma. TA suppressed the levels of Th2 cytokines (IL-4, IL-5 and IL-13), total IgE, OVA-specific IgE and ROS in BALF. Moreover, TA inhibited OVA-induced AHR to inhaled methacholine, attenuated mucus hypersecretion and reduced inflammatory cell infiltration into lung tissue. Before investigating the effects of TA (10 and 30 mg/kg) in a mouse model of allergic asthma, we studied preliminarily the dose effects of TA (5, 10, 30 and 50 mg/kg) on IgE in serum to confirm the optimal dosages and dose dependent effect of TA (data not shown). Doses of both 30 and 50 mg/kg were effective, and the lower dosage was chosen for the principal study. AHR is an important response of asthma that causes variable levels of airway obstruction [11] and is closely related

to the IgE-mediated allergic response. Th2 cytokines, including IL-4 and IL-13, contribute to AHR [22]. Data from our study showed that TA significantly inhibits the OVA-induced AHR in response to inhaled methacholine.

Cytokines released from Th2 cells are IL-4, IL-5, IL-6, IL-9, and IL-13, and those from Th1 cells are IL-2, IL-12, IFN- γ , and TNF- α [31,32]. In the initiation and progression of asthma, increased levels of Th2 cytokines, particularly IL-4, IL-5, and IL-13, are associated with inflammatory and allergic responses, since they enhance the production of IgE [23]. Specifically, IL-4 and IL-13 play critical roles in eosinophil infiltration into lung tissue and stimulate mucus hypersecretion and AHR [22,24]. Moreover, IL-5 is important for activation and survival of eosinophils as well as development of AHR. TNF- α is also an important chemoattractant for the recruitment of eosinophils into the lungs [33,34]. It is also a potent modulator of immune and inflammatory response. In our experiments, TA induced a reduction in the levels of IL-4, IL-5, IL-13, TNF- α and IgE, consistent with the inflammatory cell count in BALF. However, the level of IFN- γ was unaffected by 10 or 30 mg/kg TA (data not shown). These findings indicate that TA acts through suppression both Th2 and Th1 cytokines responsible for eosinophil recruitment into lung tissue.

Histopathological alterations, such as goblet cell hyperplasia, mucus hypersecretion, and infiltration of inflammatory cells in lung tissue, are observed in asthmatic conditions [11,18], which are closely related to the Th2 cytokine and IgE levels [25]. According to previous studies [10,18], IL-4 and IL-13 play critical roles in IgE switching within B cells, which triggers mucus hypersecretion and goblet cell hyperplasia. Similar alterations, including histopathological changes in lung tissue and increased IgE levels in BALF of OVA-induced mice, were observed in the present study. However, the extent of these alterations was significantly decreased in TA-treated mice, similar to that observed in montelukast-treated mice used as the positive control group. Our results are in agreement with data obtained for Th2 cytokines in BALF. Based on these findings, we propose that TA attenuates the inflammatory response in OVA-induced allergic asthma via modulation of Th2 cytokines.

Recent studies have shown that inflammatory responses are triggered via production of ROS in allergic asthma. Inflammatory cells are capable of generating ROS, and elevated numbers are produced under asthmatic conditions, compared to normal conditions [10,26]. Overproduction of ROS damages cell membrane phospholipids and causes impairment of membrane function, resulting in cell and tissue injury [23,27]. Thus, reduction of ROS production may have potential benefits in the treatment of lung inflammatory diseases. DCF fluorescence data support antioxidant effects of TA in the OVA-induced murine asthma model. BALF of OVA-induced mice contained markedly increased ROS levels. However, in TA-treated mice, ROS levels in BALF were significantly reduced, compared with those in OVA-induced mice. These findings clearly suggest that TA inhibits ROS-mediated airway inflammation in lung inflammatory disease.

NF- κ B plays a pivotal role in the immune and inflammatory responses, including those of asthma. In the OVA-sensitized and -challenged model, total lung extracts from Brown-Norway rats

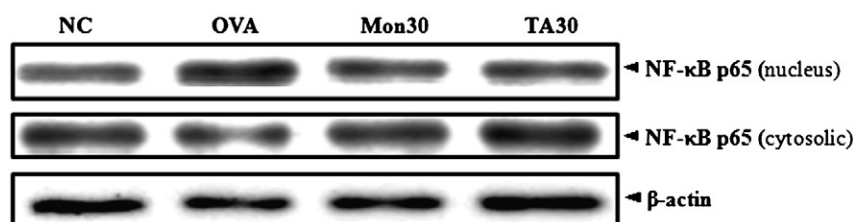


Fig. 8. Effect of TA on NF- κ B activity in lung tissue. NF- κ B p65 protein levels were measured 48 h after the last challenge in NC, normal control mice (PBS only); OVA, OVA-induced mice; TA30, tiarellic acid (30 mg/kg) + OVA-induced mice; Mon30, Montelukast (30 mg/kg) + OVA-induced mice.

showed enhanced NF- κ B activity [29]. NF- κ B levels in nuclear protein extracts from lung tissues are substantially increased in OVA-induced mouse models of asthma. Activated NF- κ B induces transcription of a variety of inflammatory genes that are abnormally expressed in asthma models [30]. Our results confirm that TA inhibits NF- κ B activity, thus preventing OVA-induced translocation of transcription factors into the nucleus. We thus suggest that observed decrease in NF- κ B activity may explain the therapeutic effect of TA.

In conclusion, to our knowledge, the current study is the first to provide experimental evidence that TA inhibits OVA-induced airway inflammation in a murine model of asthma. Administration of TA led to significant suppression of Th1 and Th2-type cytokine and IgE levels and inhibition of the recruitment of eosinophils and mucus overproduction. In addition, TA attenuated ovalbumin-induced increases in NF- κ B activity in lungs. Moreover, TA suppressed ROS generation in the OVA-induced airway inflammation reaction. Our results support the utility of TA as an effective therapeutic agent for allergic asthma.

Acknowledgments

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