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Inhibitory effects of L-theanine on airway inflammation in ovalbumin-induced allergic asthma

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ABSTRACT

L-theanine, a water-soluble amino acid isolated from green tea (*Camellia sinensis*), has anti-inflammatory activity, antioxidative properties, and hepatoprotective effects. However, the anti-allergic effect of L-theanine and its underlying molecular mechanisms have not been elucidated. In this study, we investigated the protective effects of L-theanine on asthmatic responses, particularly airway inflammation and oxidative stress modulation in an ovalbumin (OVA)-induced murine model of asthma. Treatment with L-theanine dramatically attenuated the extensive trafficking of inflammatory cells into bronchoalveolar lavage fluid (BALF). Histological studies revealed that L-theanine significantly inhibited OVA-induced mucus production and inflammatory cell infiltration in the respiratory tract and blood vessels. L-theanine administration also significantly decreased the production of IgE, monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-4, IL-5, IL-13, tumor necrosis factor-alpha (TNF-α), and interferon-gamma in BALF. The lung weight decreased with L-theanine administration. L-theanine also markedly attenuated the OVA-induced generation of reactive oxygen species and the activation of nuclear factor kappa B (NF-κB) and matrix metalloprotease-9 in BALF. Moreover, L-theanine reduced the TNF-α-induced NF-κB activation in A549 cells. Together, these results suggest that L-theanine alleviates airway inflammation in asthma, which likely occurs via the oxidative stress-responsive NF-κB pathway, highlighting its potential as a useful therapeutic agent for asthma management.

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1. Introduction

Asthma is a chronic inflammatory, allergic disorder of the airways (Poon et al., 2012). In asthma patients, smooth-muscle hyperplasia and persistent inflammation cause respiratory tract narrowing and thickening (Agrawal and Shao, 2010). In the pathogenesis of allergic asthma, exposure to allergens (e.g., harmful inhaled microorganisms, chemicals, and particles) induces various inflammatory responses, including an overproduction of reactive oxygen species (ROS) and activation of nuclear factor (NF)-κB and matrix metalloproteinase (MMP)-9, resulting in asthmatic responses in the airway (Holgate, 2008; Mehta et al., 2009). Inflammatory mediators that are involved in inflammatory cell recruitment, including macrophages, mast cells, lymphocytes, and proinflammatory cytokines (i.e., interleukin (IL)-4, IL-5, and IL-13) play an important role in airway asthmatic responses (Brightling et al., 2002; Holgate, 2008; Mehta et al., 2009; Nakajima and Takatsu, 2007).

T helper (Th) cells have an important role in the inflammatory response via the release of cytokines such as IL-12 and interferon-gamma (IFN-γ), which are involved in allergic airway inflammation

Abbreviations: BALF, bronchoalveolar lavage fluid; IgE, immunoglobulin E; INF-γ, interferon-gamma; iNOS, inducible nitric oxide synthase; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metalloproteinase-9; NF-κB, nuclear factor kappa B; OVA, ovalbumin; ROS, reactive oxygen species; Th, T helper; TNF-α, tumor necrosis factor-alpha.

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(Brightling et al., 2002; Nakajima and Takatsu, 2007). Allergic airway inflammation in asthma is associated with Th2-related responses, such as the accumulation of eosinophils, induction of cytokines particularly IL-4, IL-5, and IL-13, and production of immunoglobulin (Ig) E (Agrawal and Shao, 2010; Brightling et al., 2002; Nakajima and Takatsu, 2007). Both IL-4 and IL-13 promote acute inflammatory processes and underlying structural changes in the respiratory tract (Brightling et al., 2002; Nakajima and Takatsu, 2007). In addition, activation of Th2 cytokines has been reported to induce the secretion of IgE from B cells and the replacement of leukocytes in the lung tissue (Yuk et al., 2007). These inferences make Th2 cells a fascinating target for the improvement of asthma symptoms.

Airway inflammation results in the upregulation of a large variety of ROS, thereby causing oxidative damage to the airways. Oxidative stress can initiate and propagate inflammation, highlighting the importance of pro-oxidants in inflammatory airway disorders (Hoshino et al., 2008). Eosinophils are well known as the key effector cells in inflammatory airway disorders through the release of ROS and specific granules (Corry, 2002; Rahman et al., 1996). Despite the increasing prevalence of inflammatory airway disorders, their pathophysiology have not been fully characterized, and the current treatments are not sufficient. Asthma patients are treated with antihistamines, immunosuppressants, and bronchodilators. However, the use of these drugs is restricted because of their side effects.

Medicinal plants provide an extensive resource in the search for effective medical treatments with a low risk of adverse effects. Recently, increased attention has been paid towards the exploration of herbal preparations for the application to respiratory disease, including airway inflammation, asthma, cystic fibrosis, and chronic bronchitis (Choi et al., 2015; Shin et al., 2013; Sun et al., 2013). L-theanine (*N*-ethyl-L-glutamine), a unique non-protein amino acid found only in green tea (*Camellia sinensis*) and comprising 1–2% of the dry weight of the leaf, has antioxidative properties (Li et al., 2012), as well as immune modulating (Li et al., 2016), hepatoprotective (Pérez-Vargas et al., 2016), and neuroprotective effects (Kim et al., 2009). Furthermore, L-theanine has been shown to markedly decrease nitric oxide production via the downregulation of inducible nitric oxide synthase (iNOS) in glutamate-induced SH-SY5Y cells (Di et al., 2010). Liu et al. (2009) proposed that L-theanine attenuates β-amyloid-induced neurotoxicity through a reduction in ROS production and inactivation of the NF-κB pathways, and inhibits the growth of human leukemia and lung cancer cells. However, the antiallergic effects of L-theanine in airway inflammation have not been studied. Therefore, we examined the effects of L-theanine on ovalbumin (OVA)-induced airway inflammation in asthma and the potential mechanisms. Our data clearly suggest that L-theanine could ameliorate airway inflammation by inhibiting pro-oxidant production via inactivation of the NF-κB pathway.

2. Materials and methods

2.1. Materials

L-theanine (Fig. 1A) was purchased from TCI (Tokyo Chemical Industry co., Ltd. Tokyo, Japan). Aluminum hydroxide gel, chicken egg OVA (Grade II), phosphate-buffered saline (PBS), aluminum hydroxide [$Al(OH)_3$], TNF-α, and Giemsa solution were obtained from Sigma Chemical Co. (St. Louis, MO, USA). 2′,7′-Dichlorodihydrofluorescein diacetate (DCFDA) was purchased from Molecular Probes (Eugene, OR, USA). Enzyme-linked immunosorbent assay (ELISA) kits were purchased from BD Biosciences (San Diego, CA, USA) and R&D Systems (Minneapolis, MN, USA). NF-κB p65, phospho-NF-κB p65, β-actin, Lamin B1, and HRP-conjugated *anti*-IgG secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals and solvents were of the highest grade commercially available.

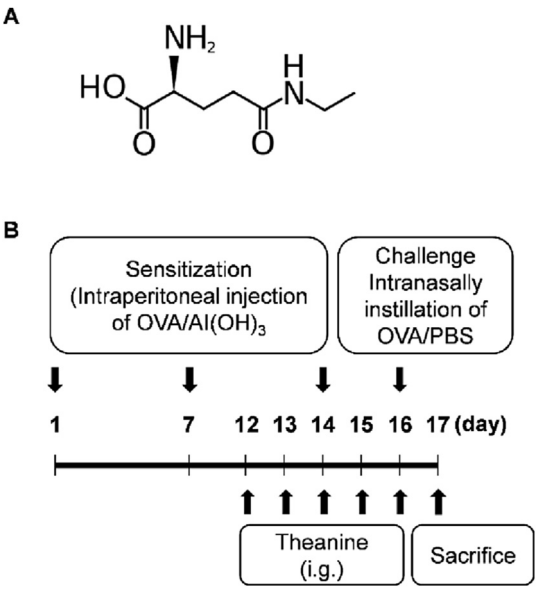

Fig. 1. (A) Chemical structure of L-theanine. (B) Schematic diagram of the experimental protocol in mice. The mice were immunized on days 0, 7, and 14 via an intraperitoneal injection of 50 μg of chicken ovalbumin (OVA) emulsified with 1 mg of aluminum hydroxide [$Al(OH)_3$] in 100 μL PBS. L-theanine was intragastrically administered to the mice at doses of 10, 50, and 100 mg/kg/day. The animals were challenged with OVA on the final day by inhalation of 1-mg/mL OVA in PBS.

2.2. Animals and OVA-induced model of asthma

Institute of Cancer Research (ICR) mice (female, 6-week-old) were obtained from Dae Han Bio Link Co., LTD (Chungbuk, Korea) and used after 1 week of acclimatization. The animals were maintained under specific pathogen-free conditions at our animal breeding facility. All experimental protocols involving the use of animals were performed according to the rules and regulations of the Animal Ethics Committee, Chungnam National University (Daejeon, Korea). The asthma model was established according to Choi et al. (2015). A schematic diagram of the treatment schedule is shown in Fig. 1B. ICR mice (6 per group) were sensitized on days 1, 7, and 14 by an intraperitoneal (i.p.) injection of 50 μg of chicken OVA emulsified in 1 mg of [$Al(OH)_3$] adjuvant, in a total volume of 100 μL of PBS (pH 7.4). The control groups ($n = 6$) were treated with PBS instead of OVA (intragastrically; i.g.). The ICR mice were i.g. administered 10, 50, or 100 mg/kg/day (in 100 μL) L-theanine dissolved in PBS daily consecutively from days 12–16. Mice were exposed to a 1% (w/v) OVA solution in PBS for 20 min by using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan) on day 16 after initial sensitization. Animals were sacrificed 24 h after the last challenge (day 17) to characterize the suppressive effects of L-theanine (Fig. 1B).

2.3. Bronchoalveolar lavage (BAL) fluid collection and leukocyte count

The mice were anesthetized by an intraperitoneal injection (0.020 mL/g weight) of 2.5% tribromoethanol (Avertin) (Sigma-Aldrich, UK, USA) 24 h after the final challenge, and a tracheostomy was performed. BALF was collected by flushing 0.5 mL of ice-cold

PBS into the lungs three times, centrifuged at 400 × g for 5 min at 4 °C, and the supernatants were stored at −80 °C for an analysis of cytokine concentrations. The cell pellet from BALF was resuspended in 100 μL of PBS. The total cell number was counted using a cytospin apparatus, and a differential cell count was performed after staining with Giemsa solution.

2.4. Measurement of proinflammatory cytokine production

The levels of IFN-γ, TNF-α, and IL-13 were measured in BALF using an R&D Systems ELISA kit. The concentrations of MCP-1, IgE, IL-4, and IL-5, were measured in BALF using a commercial BD Bioscience ELISA kit.

2.5. Measurement of intracellular ROS

To evaluate ROS production, cells from BALF were treated with 25-μM 2′,7′-dichlorofluorescein diacetate (DCFDA) for 20 min at 37 °C. Intracellular ROS was analyzed using a fluorescence plate reader (Varioskan, Thermo Electron Co.).

2.6. Western blot analysis

To obtain the extracts of lung proteins, the lung tissue was homogenized using a homogenizer in lysis buffer containing a protease inhibitor cocktail (Sigma, St. Louis, MO, USA). The protein samples were separated using 10% SDS–PAGE. Immunoblots were probed with *anti*-NF-κB p65, *anti*-phospho-NF-κB p65, *anti*-Lamin B1 or *anti*-β-actin (Santa Cruz, CA, USA) and developed using an ECL detection system (Pierce Biotechnology, Rockford, IL).

2.7. Gelatin zymography analysis

The enzymatic activity of MMP-9 was assayed by gelatin zymography. The BALF from L-theanine-treated mice was electrophoresed on a 10% SDS–PAGE gel containing 0.2% gelatin as an MMP substrate. The gel was washed with 2.5% Triton X-100 and then incubated at 37 °C for 24 h in developing buffer (50-mM Tris–HCl, pH 7.5, 150-mM NaCl, 10-mM $CaCl_2$, 0.02% NaN_3). After incubation, the gel was stained with 0.25% Coomassie Brilliant Blue G250 (Sigma Chemical Co., St. Louis, MO, USA) and then destained. MMP activity was visualized as white bands against a blue background, representing areas of proteolysis.

2.8. Histopathological studies

Lung tissue was sliced and fixed in 10% (v/v) paraformaldehyde. The tissues were paraffin-embedded, sectioned at 4-μm thickness, and stained with an H&E solution (Sigma–Aldrich) and periodic acid–Schiff (PAS) (IMEB Inc., CA, USA) to estimate the degree of mucus hypersecretion and inflammation, respectively.

2.9. Cell culture

Human lung carcinoma cell line A549 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). A549 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified chamber with 5% CO_2.

2.10. Statistical analyses

All experiments were repeated at least three times. Results reported are means ± standard error of the mean (SEM). Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by the Tukey–Kramer test, with $p < 0.05$ or $p < 0.01$ indicating significance.

3. Results

3.1. Effects of L-theanine on the number of inflammatory cells in BALF of OVA-induced airway inflammation model

To evaluate the effectiveness of L-theanine in a mouse model of asthma, OVA-induced total cells and leukocytes (i.e., macrophages, lymphocytes, basophils, and eosinophils) were counted in BALF. As shown in Fig. 2, OVA administration markedly increased the total cell count and the number of differential leukocytes in BALF, which were significantly suppressed by L-theanine compared with that reported for the vehicle control.

3.2. Histological analysis of the effects of L-theanine on airway remodeling

To investigate the extent of inflammatory cell infiltration, we performed histological analyses with the OVA-induced asthmatic airway tissues (Fig. 3). The lung weight of the OVA-challenged mice slightly increased compared to that in the negative control group. In contrast, treatment with L-theanine slightly decreased the lung weight (Fig. 3A). Our results suggest that L-theanine inhibited the OVA-induced lung weight gain in asthmatic airway tissues. Next, we examined the inhibitory effect of L-theanine on OVA-induced histopathological changes in asthmatic airway tissues. As shown in Fig. 3B, OVA-induced mice exhibited marked goblet cell hyperplasia and infiltration of inflammatory cells into the perivascular and peribronchial lesions in the lung tissue (Fig. 3B). However, compared with the OVA-induced mice, L-theanine-treated mice showed decreased goblet cell hyperplasia and inflammatory cell infiltration (Fig. 3B and C).

3.3. Effects of L-theanine on IgE levels in the serum and BALF of OVA-induced mice

Allergic airway inflammation in asthma is associated with Th2-related responses, including the production of immunoglobulin (Ig) E via the activation of mast cells (Agrawal and Shao, 2010). In the airways of allergic asthma patients, mast cells undergo degranulation and release various biological mediators, which play an important role in the progression of allergic airway inflammation (Agrawal and Shao, 2010). We determined the effectiveness of L-theanine against OVA-specific IgE secretion in the serum and BALF. As shown in Fig. 4, OVA-specific IgE secretion markedly increased in OVA-induced mice, whereas these levels significantly decreased in L-theanine-treated mice (Fig. 4A and B).

3.4. Effects of L-theanine on the level of proinflammatory cytokines and MCP-1 in the BALF of OVA-challenged mice

We examined the level of proinflammatory cytokines and MCP-1 in BALF by ELISA. The OVA-sensitized/challenged mice showed a marked elevation of TNF-α, IFN-γ, IL-4, IL-5, IL-13, and MCP-1 secretion in BALF compared with that reported for the PBS-treated controls. However, treatment with L-theanine significantly suppressed this increase (Fig. 5).

3.5. Effects of L-theanine on ROS generation and the expression of proinflammatory proteins in OVA-challenged mice and TNF-α-induced A549 cells

Oxidative stress is closely associated with allergic and

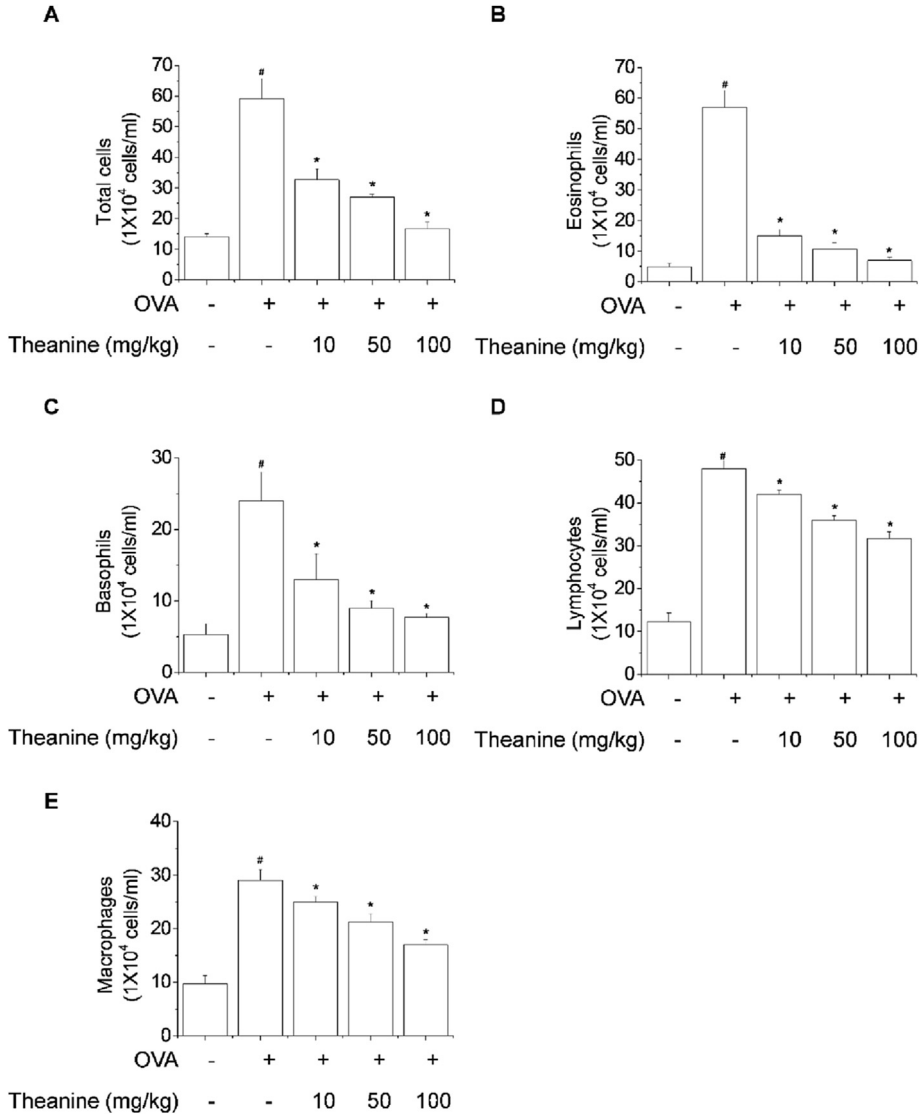

Fig. 2. Effects of theanine on OVA-induced inflammatory cell recruitment. Inflammatory cell counts were performed in bronchoalveolar lavage fluid (BALF; 0.8 mL) obtained from immunized mice 24 h after the last challenge with PBS and OVA with or without the PBS vehicle control, or L-theanine. Inflammatory cell counts were performed using standard morphological criteria via cytospin (cells per milliliter $\times$ 10^4). At least 200 cells were examined in each cytospin. The results represent the mean $\pm$ SEM (n = 6). #$P < 0.05$, significantly different from negative control. *$P < 0.05$, significantly different from the OVA-challenged group.

immunological disorders, such as asthma, atopic dermatitis, and allergic rhinitis (Corry, 2002; Hoshino et al., 2008; Rahman et al., 1996). Many studies have shown that increased ROS production in allergic respiratory diseases contributes, in part, to tissue damage at sites of inflammation (Bowler and Crapom, 2002; Hoshino et al., 2008). In this study, we examined the inhibitory effect of L-theanine on ROS production in BALF from OVA-challenged mice. As shown in Fig. 6A, OVA-challenged mice exhibited dramatically increased ROS generation in BALF compared to that observed for the control group. However, the L-theanine-treated mice exhibited dramatically decreased ROS generation in BALF compared to that reported for the OVA-induced mice. NF-κB activation plays an important role in allergic airway inflammation. Activated NF-κB by proinflammatory stimuli is translocated to the nucleus where it binds to NF-κB binding sites in the promoter regions of proinflammatory genes, and induces the transcription of proinflammatory factors, including proinflammatory cytokines and MMP-9 (Shin et al., 2013). Thus, we next examined the effect of L-theanine on the levels of the transcription factor, NF-κB p65 in a murine model of OVA-induced airway inflammation. Compared to the control groups, the positive control group showed a significant increase in the translocation of NF-κB to the nucleus in the lung tissue. In contrast, L-theanine treatment inhibited OVA-induced activation of NF-κB in a dose-dependent manner (Fig. 6B). Next, we examined the inhibitory effect of L-theanine on TNF-α-induced NF-κB activation in A549 cells. L-theanine inhibited TNF-α-induced NF-κB activation (Fig. 6C and D). Furthermore, L-theanine treatment inhibited the OVA-induced activation of MMP-9 activity in a dose-dependent manner (Fig. 6E).

4. Discussion

Allergic asthma is a chronic inflammatory disease and one of the most prevalent respiratory diseases worldwide. It is characterized by airway inflammation, airway hyper-responsiveness, and mucus hypersecretion. Thus far, a variety of drugs such as β2 adrenergic agonists, steroids, leukotriene inhibitors, and mast-cell stabilizers have been developed to treat allergic asthma. However, the use of

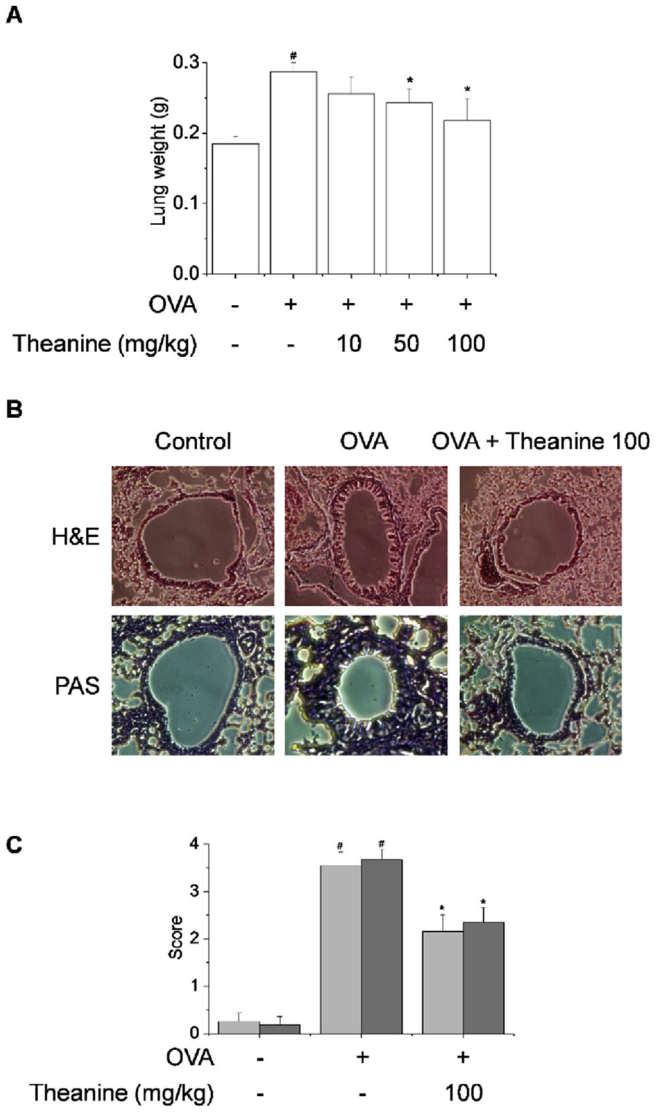

Fig. 3. L-theanine inhibits airway inflammation induced by OVA challenge. (A) Effect of L-theanine on OVA-induced lung weight. The lung weight was measured by a portable balance (Voyager® *Pro*, OHAUS). (B) Histological examination of airway inflammation in OVA-induced lung tissue by H&E and alcian blue-PAS staining. (C) Quantitative analysis of inflammatory cell infiltration and mucus production in the lung tissue. The scoring data are presented as the means ± SEM of three independent experiments. Magnification × 100; #$P < 0.05$, significantly different from the negative control. *$P < 0.05$, significantly different from the OVA-challenged group.

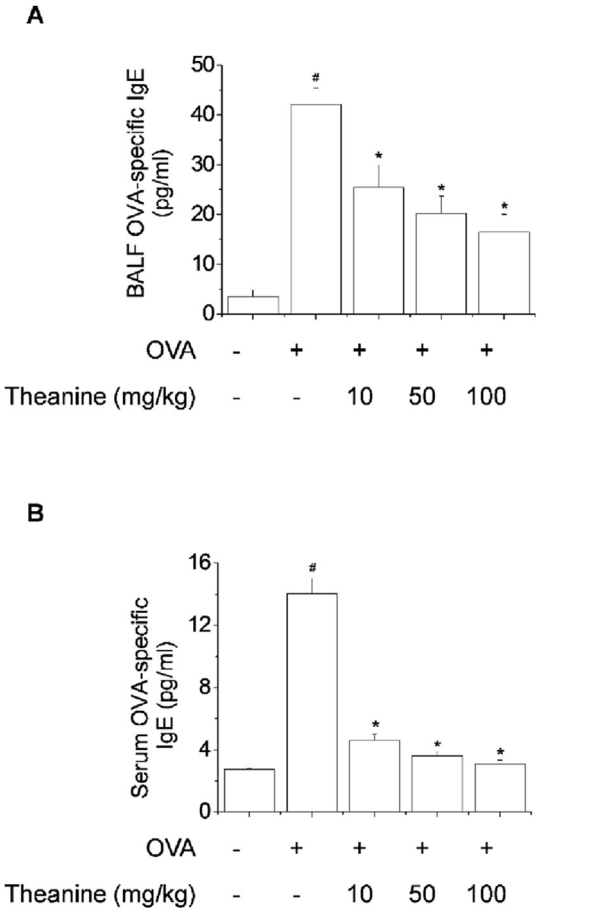

Fig. 4. Effect of L-theanine on OVA-specific IgE levels in BALF and serum. BALF was collected from OVA-challenged mice treated with or without 10, 50, and 100 mg/kg of L-theanine. The OVA-specific IgE levels in BALF (A) and serum (B) were measured using a commercial ELISA kit. Results represent the mean ± SEM (n = 6). #$P < 0.05$, significantly different from the negative control. *$P < 0.05$, significantly different from the OVA-challenged group.

these pharmacological agents is restricted because of their harmful side effects (Kayani and Shannon, 2002). Many natural agents have been used to treat allergic asthma, but their modes of action and therapeutic efficacies remain unclear. L-theanine is a biologically active ingredient in green tea and a widely used pharmacological agent in cell biology, with reported antioxidant, anti-inflammatory, relaxant, and anxiolytic properties (Kimura et al., 2007; Kim et al., 2009; Li et al., 2012, 2016). However, the effect of L-theanine on airway inflammation has not been fully characterized. In this study, we report the inhibitory effects of L-theanine on airway inflammation in OVA-challenged mice.

Respiratory diseases are caused by undesirable responses to inhaled allergens, such as harmful microorganisms, chemicals, and particles. Previous studies have shown that the underlying processes that drive and maintain the asthmatic inflammatory response include the activation of inflammatory cells and overproduction of proinflammatory cytokines, chemokines, and allergen-specific IgE (Agrawal and Shao, 2010; Ci et al., 2012; Nakajima and Takatsu, 2007). Moreover, the infiltration of multiple inflammatory cells, specifically, lymphocytes and eosinophils, into the lung tissue is a primary contributor to the progression of airway inflammation, including allergic asthma (Agrawal and Shao, 2010). In this study, we examined the inhibitory effects of L-theanine on the secretion of total cells and leukocytes in the BALF of OVA-challenged mice. The numbers of OVA-induced total cells and differential leukocytes were decreased by L-theanine treatment. In addition, a histological analysis revealed that L-theanine administration inhibited OVA-induced goblet cell hyperplasia and the infiltration of inflammatory cells into the airway tissue.

Th2 cells are the major effector cells involved in the progression of asthma (Georas et al., 2005). Activated Th2 cells secrete high levels of IL-5, IL-4, and IL-13, causing airway inflammation, including hyper-responsiveness, goblet cell hyperplasia, eosinophil infiltration, and excessive mucus secretion (Afshar et al., 2008). Furthermore, activated Th2 cells increase the secretion of IgE from B cells, and activate mast cells (Georas et al., 2005; Kim et al., 2013). Consequently, activated mast cells degranulate and release various biological mediators, which play an important role in the progression of allergic airway inflammation (Agrawal and Shao, 2010). These characteristics make Th2 cells an attractive target for the

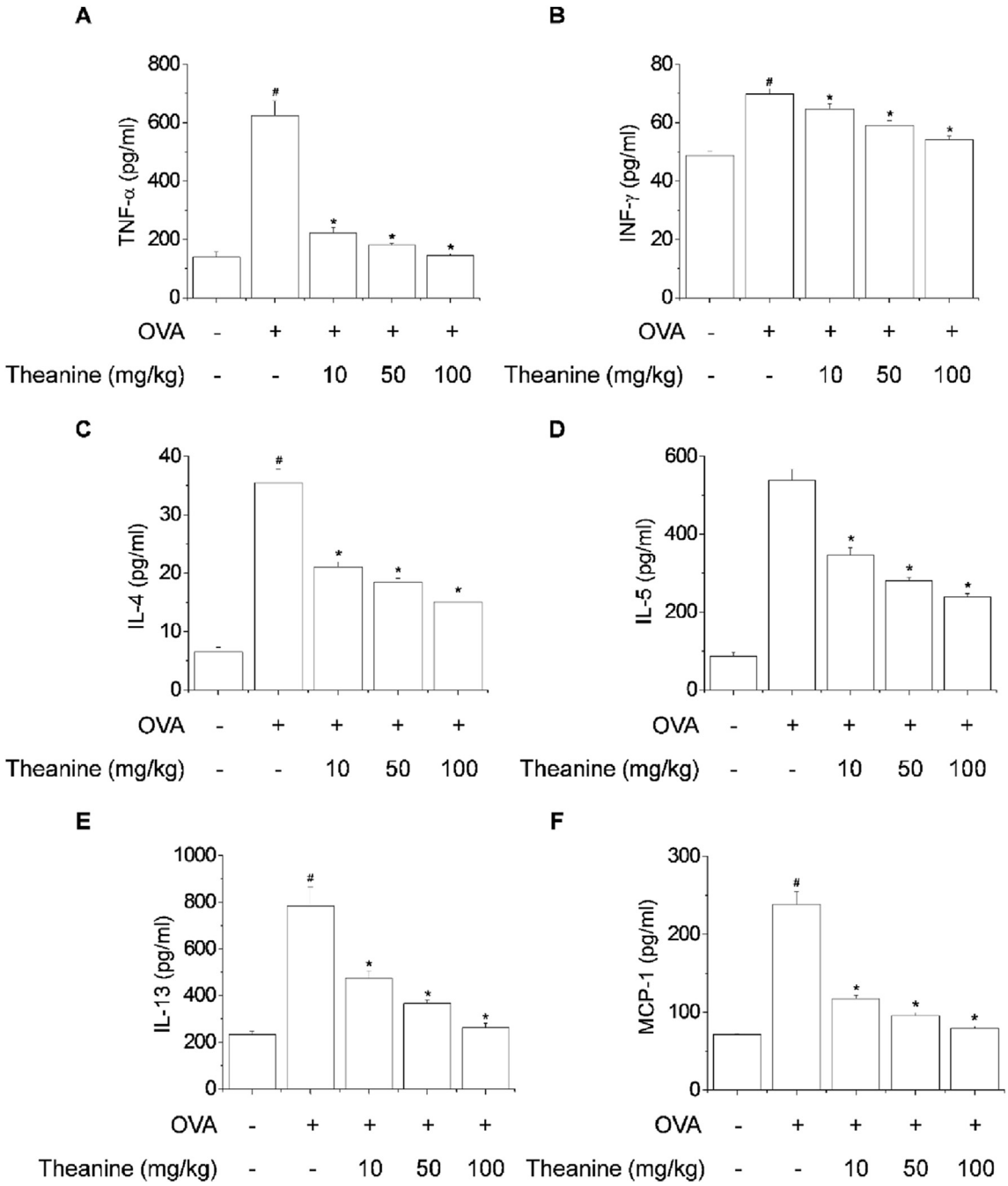

Fig. 5. L-theanine decreases the levels of TNF-α, IFN-γ, IL-4, IL-5, IL-13, and MCP-1 in BALF. The levels of cytokines and chemokines in BALF were analyzed using ELISA kits. Results are the mean ± SEM (n = 6). #$P < 0.05$, significantly different from negative control. *$P < 0.05$, significantly different from the OVA-challenged group.

amelioration of airway inflammation symptoms (Kim et al., 2013) and the overall improvement of asthma symptoms. A recent study reported that L-theanine alleviated aroclor 1254-induced neuropathological changes by inhibiting proinflammatory cytokine production (Sumathi et al., 2016). Thus, we examined the effects of L-theanine on the secretion of proinflammatory (Th1 and Th2) cytokines in OVA-challenged mice. The OVA-induced secretion of Th2 (IL-4, IL-5, and IL-13) and Th1 cytokines (TNF-α and IFN-γ), decreased following L-theanine treatment. We also confirmed that L-theanine administration decreased OVA-specific IgE production in the serum and BALF, confirming the previously reported inhibitory effects of L-theanine on mast cell-mediated anaphylactic reactions (Kim et al., 2012).

Recent studies have shown that NF-κB is associated with ROS-mediated regulation of gene expression in various inflammatory diseases, including allergic asthma (Rahman, 2003). Activated NF-κB then binds to the promoter regions of inflammatory genes, including cytokines, chemokines, adhesion molecules, and MMP-9 (Barnes and Karin, 1997). A previous study reported that L-theanine attenuated β-amyloid-induced neuronal cell death via inactivation of the NF-κB pathways (Kim et al., 2009). Liu et al. (2009) also reported that L-theanine decreased ethanol-triggered ROS production in L02 cells. In our study, L-theanine decreased ROS production and NF-κB activation induced by OVA treatment. Also, L-theanine

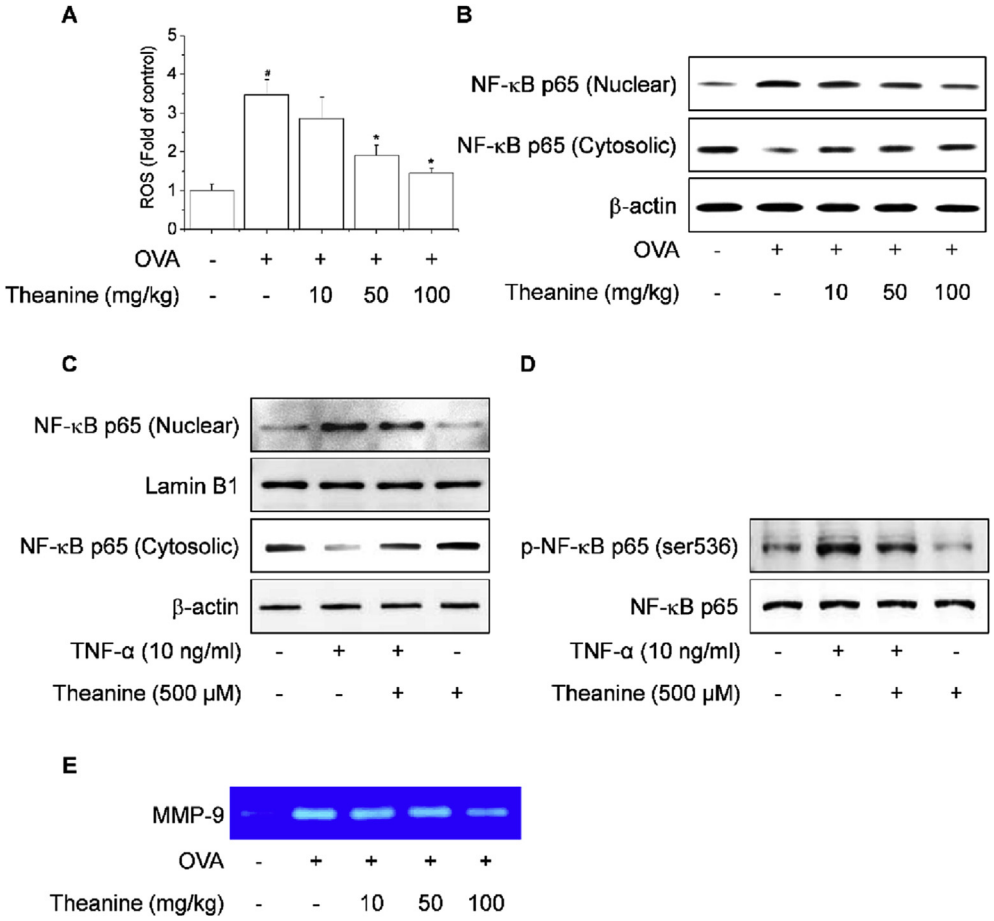

Fig. 6. L-theanine inhibits ROS generation and the expression of proinflammatory proteins in OVA-challenged mice. (A) Cells in BALF were washed with PBS and treated with 25-μM DCFDA for 20 min. Intracellular ROS were measured using a fluorescence plate reader. (B) The level of NF-κB p65 protein in both the nuclei and cytosol was measured using specific antibodies. (C and D) Effects of L-theanine on TNF-α-induced NF-κB activation in A549 cells. (C) Cells were treated with 500 μM L-theanine for 12 h and then stimulated with 10 ng/mL TNF-α for 6 h. The level of NF-κB p65 protein in both the nuclei and cytosol was measured using specific antibodies. (D) Cells were treated with 500 μM L-theanine for 12 h and then stimulated with 10 ng/mL TNF-α for 30 min. Total protein was analyzed by western blotting using antibodies against phospho-NF-κB p65 and NF-κB p65. (E) MMP-9 activity in BALF was analyzed by gelatin zymography. #$P < 0.05$, significantly different from negative control. *$P < 0.05$, significantly different from the OVA-challenged group.

inhibited TNF-α-induced NF-κB activation in A549 cells. In addition, L-theanine significantly suppressed MMP-9 activity in OVA-challenged mice via reduction of oxidative stress.

In summary, our study showed that L-theanine effectively attenuated airway inflammation in the OVA-sensitized lungs of mice. In addition, treatment with L-theanine resulted in a significant reduction of ROS generation and proinflammatory related proteins, including NF-κB and MMP-9. These results suggest that L-theanine may have therapeutic potential for effectively inhibiting the progression of airway inflammation in asthma.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Transparency document

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References

Afshar, R., Medoff, B.D., Luster, A.D., 2008. Allergic asthma: a tale of many T cells. Clin. Exp. Allergy 38, 1847–1857.
Agrawal, D.K., Shao, Z., 2010. Pathogenesis of allergic airway inflammation. Curr. Allergy Asthma Rep. 10, 39–48.
Barnes, P.J., Karin, M., 1997. Nuclear factor-kappa B: a pivotal transcription factor in chronic inflammatory diseases. New Engl. J. Med. 336, 1066–1071.
Bowler, R.P., Crapom, J.D., 2002. Oxidative stress in allergic respiratory diseases. J. Allergy Clin. Immunol. 110, 349–356.
Brightling, C.E., F.A. Symon, S.S., Bradding, P., Pavord, I.D., Wardlaw, A.J., 2002. TH2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis. J. Allergy Clin. Immunol. 110, 899–905.
Choi, J.H., Jin, S.W., Kim, H.G., Choi, C.Y., Lee, H.S., Ryu, S.Y., Chung, Y.C., Hwang, Y.J., Um, Y.J., Jeong, T.C., Jeong, H.G., 2015. Saponins, especially platyconic acid A, from Platycodon grandiflorum reduce airway inflammation in ovalbumin-induced mice and PMA-exposed A549 cells. J. Agric. Food Chem. 63, 1468–1476.
Ci, X., Chu, X., Wei, M., Yang, X., Cai, Q., Deng, X., 2012. Different effects of farrerol on an OVA-induced allergic asthma and LPS-induced acute lung injury. PLoS One 7, e34634.
Corry, D.B., 2002. Emerging immune targets for the therapy of allergic asthma. Nat. Rev. Drug Discov. 1, 55–64.
Di, X., Yan, J., Zhao, Y., Zhang, J., Shi, Z., Chang, Y., Zhao, B., 2010. L-theanine protects the APP (Swedish mutation) transgenic SH-SY5Y cell against glutamate-

induced excitotoxicity via inhibition of the NMDA receptor pathway. Neuroscience 168, 778–786.
Georas, S.N., Guo, J., De Fanis, U., Casolaro, V., 2005. T-helper cell type-2 regulation in allergic disease. Eur. Respir. J. 26, 1119–1137.
Holgate, S.T., 2008. The airway epithelium is central to the pathogenesis of asthma. Allergol. Int. 57, 1–10.
Hoshino, T., Okamoto, M., Takei, S., Sakazaki, Y., Iwanaga, T., Aizawa, H., 2008. Redox-regulated mechanisms in asthma. Antioxid. Redox Signal 10, 769–783.
Kayani, S., Shannon, D.C., 2002. Adverse behavioral effects of treatment for acute-exacerbation of asthma in children: a comparison of two doses of oral steroids. Chest 122, 624–628.
Kim, N.H., Jeong, H.J., Kim, H.M., 2012. Theanine is a candidate amino acid for pharmacological stabilization of mast cells. Amino Acids 42, 1609–1618.
Kim, T.I., Lee, Y.K., Park, S.G., Choi, I.S., Ban, J.O., Park, H.K., Nam, S.Y., Yun, Y.W., Han, S.B., Oh, K.W., Hong, J.T., 2009. l-Theanine, an amino acid in green tea, attenuates beta-amyloid-induced cognitive dysfunction and neurotoxicity: reduction in oxidative damage and inactivation of ERK/p38 kinase and NF-kappaB pathways. Free Radic. Biol. Med. 47, 1601–1610.
Kim, Y.M., Kim, Y.S., Jeon, S.G., Kim, Y.K., 2013. Immunopathogenesis of allergic asthma: more than the Th2 hypothesis. Allergy Asthma Immunol. Res. 5, 189–196.
Kimura, K., Ozeki, M., Juneja, L.R., Ohira, H., 2007. L-Theanine reduces psychological and physiological stress responses. Biol. Psychol. 74, 39–45.
Li, C., Tong, H., Yan, Q., Tang, S., Han, X., Xiao, W., Tan, Z., 2016. L-theanine improves immunity by altering TH2/TH1 cytokine balance, brain neurotransmitters, and expression of phospholipase C in rat hearts. Med. Sci. Monit. 22, 662–669.
Li, G., Ye, Y., Kang, J., Yao, X., Zhang, Y., Jiang, W., Gao, M., Dai, Y., Xin, Y., Wang, Q., Yin, Z., Luo, L., 2012. l-Theanine prevents alcoholic liver injury through enhancing the antioxidant capability of hepatocytes. Food Chem. Toxicol. 50, 363–372.
Liu, Q., Duan, H., Luan, J., Yagasaki, K., Zhang, G., 2009. Effects of theanine on growth of human lung cancer and leukemia cells as well as migration and invasion of human lung cancer cells. Cytotechnology 59, 211–217.
Mehta, A.K., Arora, N., Gaur, S.N., Singh, B.P., 2009. Choline supplementation reduces oxidative stress in mouse model of allergic airway disease. Eur. J. Clin. Invest. 39, 934–941.
Nakajima, H., Takatsu, K., 2007. Role of cytokines in allergic airway inflammation. Int. Arch. Allergy Immunol. 142, 265–273.
Pérez-Vargas, J.E., Zarco, N., Vergara, P., Shibayama, M., Segovia, J., Tsutsumi, V., Muriel, P., 2016. l-Theanine prevents carbon tetrachloride-induced liver fibrosis via inhibition of nuclear factor κB and down-regulation of transforming growth factor β and connective tissue growth factor. Hum. Exp. Toxicol. 35, 135–146.
Poon, A.H., Eidelman, D.H., Martin, J.G., Laprise, C., Hamid, Q., 2012. Pathogenesis of severe asthma. Clin. Exp. Allergy 42, 625–637.
Rahman, I., 2003. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. J. Biochem. Mol. Biol. 36, 95–109.
Rahman, I., Morrison, D., Donaldson, K., MacNee, W., 1996. Systemic oxidative stress in asthma, COPD, and smokers. Am. J. Respir. Crit. Care Med. 154, 1055–1060.
Shin, I.S., Hong, J., Jeon, C.M., Shin, N.R., Kwon, O.K., Kim, H.S., Kim, J.C., Oh, S.R., Ahn, K.S., 2013. Diallyl-disulfide, an organosulfur compound of garlic, attenuates airway inflammation via activation of the Nrf-2/HO-1 pathway and NF-kappaB suppression. Food Chem. Toxicol. 62, 506–513.
Sumathi, T., Asha, D., Nagarajan, G., Sreenivas, A., Nivedha, R., 2016. l-Theanine alleviates the neuropathological changes induced by PCB (Aroclor 1254) via inhibiting upregulation of inflammatory cytokines and oxidative stress in rat brain. Environ. Toxicol. Pharmacol. 42, 99–117.
Sun, J., Li, L., Wu, J., Liu, B., Gong, W., Lv, Y., Luo, Q., Duan, X., Dong, J., 2013. Effects of baicalin on airway remodeling in asthmatic mice. Planta Medica 79, 199–206.
Yuk, J.E., Woo, J.S., Yun, C.Y., Lee, J.S., Kim, J.H., Song, G.Y., Yang, E.J., Hur, I.K., Kim, I.S., 2007. Effects of lactose-beta-sitosterol and beta-sitosterol on ovalbumin-induced lung inflammation in actively sensitized mice. Int. Immunopharmacol. 7, 1517–1527.