Food and Chemical Toxicology 99 (2017) 162–169

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

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

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ARTICLE INFO

Article history:
Received 20 July 2016
Received in revised form
22 November 2016
Accepted 25 November 2016
Available online 29 November 2016

Keywords:
L-theanine
Ovalbumin
Cytokine
Airway inflammation
NF-κB

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.

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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http://dx.doi.org/10.1016/j.fct.2016.11.032
0278-6915/

(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.

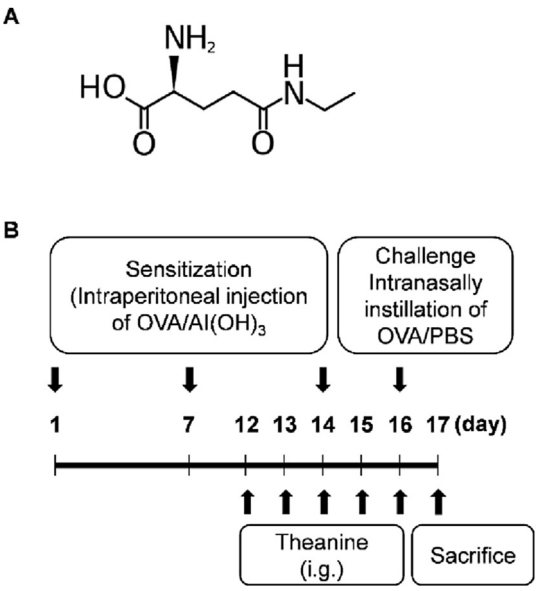

Fig. 1. (A) Chemical structure of L-theanine. (B) Schematic diagram of the experimental protocol in mice. The mice were immunized on days 0, 7, and 14 via an intraperitoneal injection of 50 μg of chicken ovalbumin (OVA) emulsified with 1 mg of aluminum hydroxide [$Al(OH)_3$] in 100 μL PBS. L-theanine 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