

Ginseng ameliorates chronic histopathologic changes in a murine model of asthma

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ABSTRACT

Currently, asthma therapies are effective in reducing inflammation but airway remodeling is poorly responsive to these agents. New therapeutic options that have fewer side effects and reverse chronic changes in the lungs are essential. This study aimed to determine the efficacy of oral administration of ginseng on lung histopathology in a murine model of chronic asthma. BALB/c mice were divided into four groups: control, placebo, ginseng, and dexamethasone. All mice except those in the control group were sensitized and challenged with ovalbumin. Then, mice in the ginseng group were given 2 gr/kg per day of ginseng and mice in the dexamethasone group received 1 mg/kg per day of dexamethasone via orogastric gavage once daily for 1 week. Lung histopathology was evaluated by using light and electron microscopy in all groups. All of the chronic changes of airways in the ginseng group were significantly ameliorated when compared with the placebo group. When compared with the dexamethasone group, the ginseng group had significantly lower numbers of mast cell count. Thicknesses of basement membrane, epithelium, and subepithelial smooth muscle were not statistically different between the ginseng and dexamethasone groups. Goblet cell numbers were much more reduced in the dexamethasone group. Ginseng is effective in resolving the established chronic histopathological changes of the lungs in the murine model of asthma.

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Key words: BALB-c mice, CAM, dexamethasone, Ginseng, histopathology, lung, mouse model of asthma, remodeling

Asthma is a chronic inflammatory disorder of the airways that is associated with recruitment of inflammatory cells and the clinical development of wheezing, shortness of breath, chest tightness, and cough.¹ Inhaled glucocorticoids, which are generally effective and well tolerated, are currently the mainstay of asthma therapy, although several side effects may occur when they are used at high doses or for a prolonged time.² The use of complementary and alternative treatments in asthma patients is increasing in recent years.³ Herbal preparations have been cited as one of the most popular complementary treatment modalities used by asthmatic patients.⁴ There are reports that 11%³ to 40%⁵ of people with asthma may use herbal remedies.

Ginseng, is a slow-growing root herb that has been used for thousands of years by practitioners of tradi-

tional Chinese medicine.⁶ The main active components of ginseng are glycosidal saponins known as ginsenosides.^{7,8} These ginsenosides have been reported to show many biological activities including anti-inflammatory activity⁹ and glucocorticoid-like effects.¹⁰ There is evidence that ginsenosides strongly increased the production of type 1 cytokines interleukin (IL)-2 and interferon (IFN) γ and decreased the production of type 2 cytokines IL-4 and IL-10 *via* modulatory cytokines, transcription factor gene expression, and the DNA binding activity of nuclear factor κ B, the production of type 2 cytokines IL-4, IL-10 by modulation cytokines and transcription factors gene expression and regulation nuclear factor- κ B DNA binding activity.¹¹ It was also shown that ginsenoside relaxes human bronchial smooth muscle presumably *via* stimulation of nitric oxide generation from airway epithelium.¹² Some studies suggested that ginseng might be a desirable agent for the correction of Th2-dominant pathological disorders such as asthma but there is no study reported in the literature evaluating the effect of ginseng on lung histopathological changes related to asthma. In this study, we investigated whether oral administration of ginseng would show an ameliorating effect on lung histopathological features and compared the effects of ginseng and dexamethasone in a murine model of asthma.

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MATERIALS AND METHODS

Experimental Animals

Pathogen-free, 6- to 8-week-old female BALB/c mice, weighing 28–30 g, were purchased from Ege University Faculty of Veterinary Laboratories (Izmir, Turkey) and maintained in a pathogen-free laboratory of Dokuz Eylul University. They were kept in hygienic macroline cages and in air-conditioned rooms with a 12-hour light/12-hour dark cycle. The Animal Ethics Committee of Dokuz Eylul University approved the experimental procedures.

Study Groups

Twenty-eight mice were divided into four groups: control group ($n = 7$), placebo group ($n = 7$), ginseng group ($n = 7$), and dexamethasone group ($n = 7$).

Establishment of Chronic Asthma Model

BALB/c mice are high responders to ovalbumin.¹³ The mice in the study group and placebo group were sensitized *via* two i.p. injections of 10 μ g/0.1 mL of chicken egg albumin (ovalbumin, grade V, $\geq 98\%$ pure; Sigma, St. Louis, MO) with alum as an adjuvant on days 0 and 14 of the experiment. The mice in the treatment and placebo group were then exposed to aerosolized ovalbumin for 30 minutes/day on 3 days of the week for 8 weeks beginning from the 21st day of the study. The mice in the control group received normal saline with alum i.p. on days 0 and 14 of the experiment and aerosolized saline without alum for 30 minutes/day on 3 days of the week for 8 weeks beginning from the 21st day of the study.^{13,14} Exposures were performed in a whole body inhalation exposure system. A solution of 2.5% ovalbumin in normal saline was aerosolised by delivery of compressed air to a sidestream jet nebulizer and injected into a chamber.

Study Drugs

BALB/c mice were given 2 gr/kg per day of ginseng by orogastric gavage once daily for 7 days. This dosage was adapted from another study conducted with BALB/c mice again.¹⁵ Dexamethasone was also given to the mice with a dose of 1 mg/kg per day once daily by orogastric gavage for 7 days. The ginseng used was Panax ginseng from the Araliaceae family. Ginseng was purchased from the market as standardized tablet form (Gerimax, Dansk Droge A/S, Ishøj, Denmark). The ginseng product that we used in the present study (Gerimax ginseng) included seven different ginsenosides. The type and percentages of ginsenosides in this product were Rg1, 17%; Re, 9%; Rf, 3%; Rb1, 26%; Rb2, 16%; Rc, 13%; and Rd, 16%. Ginseng tablets were powdered and powdered tablets were extracted with distilled water. The combined water phases were fil-

tered and distilled *in vacuo* to yield the extract. Gerimax ginseng powder contained 8.5% (w/w) of ginsenosides. The ginseng product used in the study was produced in accordance with Hazard Analysis and Critical Control Point. The manufacturer of ginseng follows standards established by the Danish and European Union legislation to avoid substandard conditions such as plant substitutions, toxic or stimulant adulterations, absence of ginsenosides, mixing species, and labeling errors.

Histopathological Analysis

Animals were killed by an overdose of ketamine 24 hours after the last inhalational exposure and histopathological specimens were collected. Samples were fixed in 10% formalin for light microscopic evaluation. Serial sections cut at 5 μ m were stained with hematoxylin and eosin (for routine histopathological examination), with toluidine blue (for enumeration of mast cells), and with periodic acid-Schiff (for enumeration of goblet cells). Photomicrographs were taken by a TK-890-E camera (JVC, Yokohama, Japan), which was adapted on an Olympus BH-2 RFCA model microscope (Olympus Optical Co., Ltd, Tokyo, Japan). Measurements of the thicknesses of epithelium and subepithelial smooth muscle layers and the number of goblet cells and mast cells were recorded. The histological analysis was performed with a UTHSCSA Image Tool for Windows Version 3.00 software (The University of Texas Health Center, San Antonio, TX).

Samples were fixed in 2% glutaraldehyde for electron microscopic evaluation. Tissues were embedded in EPON after the follow-up process of electron microscopic evaluation. Respiratory tracts were marked from the semithin sections. Ultrathin sections were stained with uranyl acetate and lead citrate. A Libra 120 electron microscope (Carl Zeiss, Oberkochen, Germany) was used for this evaluation. Basal membrane thicknesses of samples of respiratory epithelium were examined with electron microscopy by using ITEM Version 5.0 (Olympus Soft Imaging Solutions GmbH, Münster, Germany [Copyright 1986–2007]) program.

Statistical Analysis

The SPSS 11 package program (SPSS, Inc., Chicago, IL) was used in the statistical analysis. The comparisons between all groups were conducted by using Kruskal-Wallis method. When differences were statistically significant, the Mann-Whitney *U* test was used for group comparisons. A value of $p < 0.05$ was considered statistically significant.

RESULTS

When compared with the control group, the asthma group (placebo group) had significantly higher num-

Table 1 Comparison between control group and asthmatic mice (placebo group)

	Control Group Mean (Range; <i>n</i> = 7)	Placebo Group Mean (Range; <i>n</i> = 7)	<i>p</i> Value
Basement membrane thickness (nm)	308.16 (245.19–389.47)	655.13 (507.36–771.02)	0.000
Epithelium thickness (μm)	19.58 (16.79–21.15)	32.36 (21.33–43.98)	0.000
Subepithelial smooth muscle layer thickness (μm)	7.27 (4.92–9.8)	10.55 (7.71–13.03)	0.000
Goblet cell number/100 μm	0.33 (0–0.36)	5.29 (2.69–13.64)	0.000
Mast cell number (<i>n</i>)	7 (6–8)	18 (15–21)	0.000

Table 2 Comparison between placebo and ginseng group

	Placebo Group Mean (Range; <i>n</i> = 7)	Ginseng Group Mean (Range; <i>n</i> = 7)	<i>p</i> Value
Basement membrane thickness (nm)	655.13 (507.36–771.02)	422.75 (312.74–595.43)	0.000
Epithelium thickness (μm)	32.36 (21.33–43.98)	22.0 (14.3–28.8)	0.004
Subepithelial smooth muscle layer thickness (μm)	10.55 (7.71–13.03)	5.56 (5.38–5.7)	0.000
Goblet cell number/100 μm	5.29 (2.69–13.64)	0.94 (0.66–1.86)	0.000
Mast cell number (<i>n</i>)	18 (15–21)	6 (4–8)	0.000

Table 3 Comparison between ginseng and dexamethasone group

	Ginseng Group Mean (Range; <i>n</i> = 7)	Dexamethasone Group Mean (Range; <i>n</i> = 7)	<i>p</i> Value
Basement membrane thickness (nm)	422.75 (312.74–595.43)	399.15 (272.58–600.01)	0.330
Epithelium thickness (μm)	22.0 (14.3–28.8)	22.44 (17.44–26.72)	0.972
Subepithelial smooth muscle layer thickness (μm)	5.56 (5.38–5.7)	5.22 (3.26–6.74)	0.123
Goblet cell number/100 μm	0.94 (0.66–1.86)	0.68 (0–2.84)	0.000
Mast cell number (<i>n</i>)	6 (4–8)	11.5 (8–15)	0.002

bers of goblet and mast cells with increased thicknesses of epithelium, basement membrane, and subepithelial smooth muscle layers. These results revealed that the asthma model was successfully established. Table 1 presents the mean and range values of histopathological parameters evaluated.

All airway histopathology parameters of the ginseng group were statistically significantly ameliorated when compared with the placebo group (Table 2). When compared with the dexamethasone group, the ginseng group had significantly lower numbers of mast cell count. Thicknesses of basement membrane, epithelium, and subepithelial smooth muscle layer were not statistically different between the ginseng and dexamethasone groups. Goblet cell numbers were much more reduced in the dexamethasone group (Table 3). Histopathological views of the airways are shown in Fig. 1.

DISCUSSION

Asthma is a chronic airway disease characterized by reversible airway obstruction, airway inflammation, and remodeling.¹⁶ Airway inflammation is the key factor in the pathogenesis of asthma and current strategies for the management focus on suppressing airway inflammation.^{17–19} Airway remodeling is a potentially important consequence of asthma.^{19,20} Although current asthma therapies are effective in reducing inflammation, airway remodeling is poorly responsive to current therapies.²¹ Thus, new approaches that have less side effects and reverse chronic changes in lungs are essential. Herbal therapies are one of the most popular alternative treatment modalities used by asthmatic patients. Although the anti-inflammatory properties of the herbal therapies are reported many times, their effects on lung histopathology and remodeling has

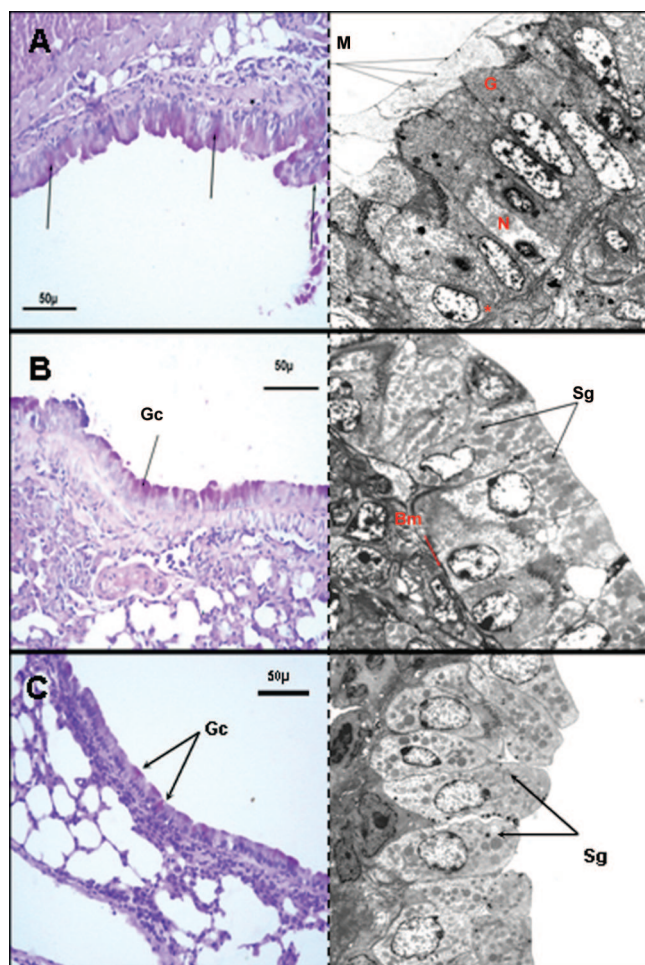


Figure 1. Light and electron microscopic findings of airways. (A) Placebo group—In the asthma group, light microscopic findings revealed irregular respiratory epithelium, some of the cell nucleuses were necrotic, and degenerative cytoplasm were seen. There were many goblet cells (arrows) in the airway section of the mouse with chronic asthma and thickness of the smooth muscle (*) was increased. In electron microscopy, degranulation was seen in the apical part of the Goblet cells (G). The surface of the epithelium was covered with mucus (M). Basement membrane, *; nucleus of epithelial cell without cilia, N. (B) Ginseng group—In light microscopy, respiratory epithelium was seen as normal. Thicknesses of smooth muscle and epithelium were decreased (periodic acid—Schiff [PAS], +; goblet cells, Gc). In electron microscopy, epithelium and surrounding structures appeared normal. Secretory granules (Sg) in apical cytoplasm of goblet cells. Basement membrane was regular (Bm). (C) Dexamethasone group—In light microscopy, respiratory epithelium was regular, number of goblet cells and thickness of smooth muscle layer were decreased. In electron microscopy, epithelial cells were in secretion phase; apical cytoplasm were full with secretory granules (Sg). Subepithelial structures appeared normal.

rarely been studied.²² Thus, we aimed to investigate the efficacy of ginseng, especially on remodeling in a murine model of chronic asthma.

Ginseng is a smooth, herbaceous, perennial member of the ivy family Araliaceae. Ginseng has been used for thousands of years in traditional Chinese medicine to treat many different diseases. The applicable part of panax ginseng is the root. Panax ginseng contains several active constituents. The constituents thought to be of most importance are triterpenoid saponins referred to collectively as ginsenosides.⁸ According to the results of *in vitro* and *in vivo* studies, ginseng can increase antitumor activity, improve cardiovascular function due to vasodilation, and reduce platelet aggregation; can have antioxidant activity, hypoglycemic activity, and immunostimulant effect; and it can cause stimulation of the pituitary adrenocortical system.²³ Ginseng has been reported to change the circulating levels of adrenocorticotrophin hormone²⁴ and corticosterone concentrations.²⁵ Rogala *et al.* showed that *Eleutherococcus senticosus* (Siberian ginseng) enhanced the cellular response of the mouse immunologic system (chemokinetic activity of mice spleen cells) and caused a stimulatory effect on the humoral response (antibody production).²⁶ Two ginsenosides, Rb1 and Rg1, have been reported to possess glucocorticosteroid activity because of a tendency to behave as functional ligands to the glucocorticoid receptor.²⁷ Chung *et al.* reported that ginsenoside Rg1 down-regulates glucocorticoid receptor and displays synergistic effects with cAMP.²⁸ Ling *et al.* showed that ginsenosides from extract of ginseng may reverse the dexamethasone-induced down-regulation of glucocorticoid receptor and the results of this study also confirmed that ginsenoside contains glucocorticoid-like activity.²⁹ Liou *et al.* showed that mice treated with different doses of ginseng root extract for 5 consecutive days had enhanced IL-2 (Th1 type cytokine), IFN- γ (Th1 type cytokine), and IL-10 production (T regulatory 1 cell cytokine and Th2 type cytokine).¹⁵ It was also shown that extract from *Eleutherococcus senticosus* roots increased the synthesis of RANTES and inhibited the release of IL-4, IL-5, and IL-12. An inhibition at higher concentrations, switching to a stimulation at lower doses of the extract, was seen with granulocyte-colony stimulating factor, IL-6, and IL-13.³⁰ There is evidence that ginsenoside-F3 in the panax ginseng has immunoenhancing activity, increased the production of type 1 cytokines, and decreased the production of type 2 cytokines.¹¹ In our study, although the cytokine profiles of the experimental animals were not evaluated, it was shown that ginseng statistically significantly ameliorated structural changes of chronic asthma.

Generally, ginseng is considered to have a relatively low level of adverse reactions and very low incidence of toxicity has been observed in ginseng clinical trials

using well-characterized preparations,³¹ but it can rarely cause some side effects such as insomnia,³² hypertension, diarrhea, mastalgia, eruptions, and vaginal bleeding.³³ Because of drug interactions, it is contraindicated in patients taking monoamine oxidase inhibitors,³⁴ antidiabetic drugs,³⁵ anticoagulants,³⁶ estrogens, or corticosteroids.³⁷ Because it is known that ginseng should not be used with corticosteroids because of possible additive effects,³⁷ we did not give dexamethasone and ginseng together to the mice in the present study.

There were some important limitations of the study. For instance, cytokine levels have an important role in asthma pathogenesis could not be evaluated, small number of animals used in our study design and the results found in our study may not translate to positive findings in human clinical trials. Also, there is no follow-up period to see if the ameliorating effect of ginseng on lung histopathology is permanent.

In conclusion, this study has shown that administration of ginseng reversed the structural changes seen with airway inflammation and remodeling in a murine model of chronic asthma. Consequently, ginseng may be useful in the treatment of asthma. Because ginseng has not proven to be a sufficient alternative to corticosteroids, perhaps only those patients with mild asthma not requiring steroid therapy, those in whom steroids are contraindicated, or patients who refuse or can not tolerate corticosteroids would seem to be the likely target group for this therapy. Additional research is needed to clarify the mechanisms that underlie the improving effect of ginseng on lung histopathology. The optimal dosage of ginseng, route of administration, and the duration of treatment need to be answered by future research before ginseng can be accepted and incorporated into the clinical medical practice.

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