CURRENT THERAPEUTIC RESEARCH

VOLUME 72, NUMBER 6, DECEMBER 2011

Glycyrrhizin and Long-Term Histopathologic Changes in a Murine Model of Asthma

Arzu Babayigit Hocaoglu, MD[1]; Ozkan Karaman, MD[1]; Duygu Olmez Erge, MD[1]; Guven Erbil, MD, PhD[2]; Osman Yilmaz, PhD[3]; Alper Bagriyanik, MD, PhD[2]; and Nevin Uzuner, MD[1]

[1]Division of Allergy, Department of Pediatrics, Dokuz Eylul University Hospital, Izmir, Turkey; [2]Department of Histology and Embryology, Dokuz Eylul University Hospital, Izmir, Turkey; [3]Department of Multidisciplinary Animal Laboratory, Dokuz Eylul University Hospital, Izmir, Turkey

ABSTRACT

BACKGROUND: Licorice root has been widely used to treat bronchial asthma for many years. However, the effect of this herb on lung histopathologic features is not fully understood.

OBJECTIVE: In this study, we aimed to determine the effects of oral administration of glycyrrhizin, an active constituent of licorice root, on lung histopathologic features in BALB/c mice, in which the model of chronic asthma was established.

METHODS: Twenty-eight BALB/c mice were divided into 4 groups: control, placebo, dexamethasone, and glycyrrhizin. Mice in the treatment and placebo groups were sensitized with 2 intraperitoneal injections of ovalbumin and then were exposed to aerosolized ovalbumin for 30 minutes per day on 3 days each week for 8 weeks beginning on the 21st study day. In the last week of inhalational exposure, mice in the placebo group received saline and those in the treatment groups received either dexamethasone, 1 mg/kg, or glycyrrhizin, 10 mg/kg, via orogastric gavage for 7 consecutive days. Animals were humanely killed 24 hours after the last ovalbumin and drug exposure. Lung histopathologic findings were evaluated using light and electron microscopy.

RESULTS: As evaluated in the control, placebo, dexamethasone, and glycyrrhizin groups, respectively, the mean (SD) basement membrane thickness was 306.34 (36.91), 657.52 (98.99), 405.13 (96.1), and 465.01 (121.48) nm; subepithelial smooth muscle thickness was 7.22 (1.37), 11.24 (1.85), 5.62 (1.15), and 7.76 (1.11) μm; epithelium thickness was 19.48 (1.22), 41.62 (5.49), 22.59 (3.18), and 25.54 (4.68) μm; number of mast cells was 1.34 (0.19), 3.62 (0.5), 2.06 (0.77), and 2.77 (0.23)/16,400 μm^2; and number of goblet cells was 0.32 (0.1), 4.92 (0.82), 0.66 (0.06), and 0.98 (0.15)/100 μm. Evaluation of lung histopathologic features demonstrated that the chronic asthma model of mice was successfully established, with significantly higher numbers of goblet and mast cells and increased thickness of epithelium, basement membrane, and subepithelial smooth muscle layers ($P < 0.001$

Accepted for publication November 7, 2011. doi:10.1016/j.curtheres.2011.11.002

 0011-393X

for all) in the asthma group compared with in the control group. The number of goblet ($P < 0.001$) and mast ($P < 0.02$) cells and the thickness of basement membrane ($P < 0.001$), subepithelial smooth muscle layers ($P \leq 0.001$), and epithelium of the lung ($P < 0.001$) were found to be significantly lower in the glycyrrhizin group compared with in the placebo group. When the glycyrrhizin and dexamethasone groups were compared, there was no statistically significant difference between the 2 groups in the histopathologic parameters, including thickness of basement membrane ($P = 0.514$), subepithelial smooth muscle ($P = 0.054$), and epithelium ($P = 1.0$) and number of mast ($P = 0.075$) and goblet ($P = 0.988$) cells.

Conclusions: The results of this study suggest that the group receiving glycyrrhizin had amelioration of all established chronic histopathologic changes of lung in the mouse model of asthma. Further studies are needed to evaluate the efficacy of glycyrrhizin in the management of asthma. (*Curr Ther Res Clin Exp.* 2011;72: 250–261)

Key words: airway remodeling, asthma, BALB/c mice, *Glycyrrhiza glabra*, glycyrrhizin, lung histopathology.

INTRODUCTION

Asthma, the most common chronic disease in childhood, is characterized by persisting airway inflammation, which leads to remodeling of the airways.[1] Airway remodeling causes progressive structural changes in the composition, content, and organization of the cellular and molecular constituents of the airway wall.[2] These structural changes include goblet cell hyperplasia in the epithelium, mucous gland hyperplasia, reticular basement membrane thickening, increased vascularity of mucosa, and thickening of the smooth muscle layer.[3]

Because asthma has a chronic nature, long-term medication therapy is required for disease management. Although corticosteroids are still accepted as the gold standard for asthma treatment and improve asthma symptoms, they do not alter the progression of asthma or cure the disease.[4] Also, concerns about adverse effects when they are used at high doses or for a prolonged period continue to limit patient compliance.[5] The chronic nature of the disease, the lack of definitive curative therapies, and the fear of known adverse effects of current drugs encourage patients to find complementary and alternative medicines.[6] There has been huge interest in herbal medicine, and some studies show the potential anti-inflammatory role of these agents.[7] It is known that 4 of the 5 classes of drugs currently used to treat asthma—β_2 agonists, anticholinergics, methylxanthines, and cromones—have origins in herbal treatments.[8] The dramatic increase in the number of individuals with asthma has provided a new area where alternative treatment is being considered by more and more patients, but the potential for abuse and the toxicity of herbal therapy remain concerns.[9] Also, well-controlled clinical trials using herbal medicine for asthma treatment are still rare.[8,10]

Glycyrrhiza glabra, also known as licorice, is native to the Mediterranean and to certain areas of Asia. It is composed of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances.[11] Glycyrrhizin, the major active constituent of licorice root accounting for its sweet taste, is a triterpene glycoside that consists of 1 molecule of 18β-glycyrrhetinic acid and 2 molecules of glucuronic acid having the structure 18β-glycyrrhetinic acid-3-O-β-d-glucuronopyranosyl-(1→2)-β-d-glucuronide.[12,13] It has been reported to have a variety of pharmacologic activities, including anti-inflammatory, antiallergic, and antiviral activities.[14] The mechanism of action of glycyrrhizin against inflammation could be due to the corticosteroid-like structure of 18β-glycyrrhetinic acid,[15] thus mimicking the effect of cortisol by inhibiting the catalytic activity of 11β-hydroxysteroid dehydrogenase.[16] Although it has been reported that glycyrrhizin inhibits immediate airway constriction, airway hyperreactivity, lung inflammation, and infiltration of eosinophils in the airways,[17] to our knowledge, there is no study reported in the English literature evaluating the effect of this molecule on long-term structural changes in the airway and remodeling.

In the present study, we investigated whether oral administration of glycyrrhizin, a major constituent of the plant *G glabra*, would have an ameliorating effect on lung histopathologic features, especially on airway remodeling, in a murine model of chronic asthma.

MATERIALS AND METHODS

Experimental Animals

Six- to 8-week-old female BALB/c mice weighing 18 to 20 g were purchased from Bornova Veterinary Control and Research Institute (Izmir, Turkey) and were maintained in a pathogen-free animal laboratory at Dokuz Eylul University (Izmir, Turkey). They were kept in hygienic macrolene cages in air-conditioned rooms under a 12-hour light/12-hour dark cycle, with food and water ad libitum. All the animal study protocols were reviewed and approved by the Dokuz Eylul University Animal Care Committee. The 28 study mice were divided into 4 groups: controls, placebo use, dexamethasone therapy, and glycyrrhizin therapy, each including 7 mice.

Sensitization and Inhalational Exposure

BALB/c mice were used for this study because they are high responders to immunoglobulin (Ig) E.[18] Mice in the treatment and placebo groups were sensitized with 2 intraperitoneal injections of 10 μg/0.1 mL of chicken egg albumin (ovalbumin [OVA], grade V, ≥98% pure; Sigma-Aldrich Corp., St. Louis, Missouri) with alum as an adjuvant on days 0 and 14 of the experiment. Mice in the placebo and treatment groups were then exposed to aerosolized OVA for 30 minutes per day on 3 days per week for 8 weeks beginning on the 21st day of the study. Mice in the control group received normal saline with alum intraperitoneally on days 0 and 14 of the experiment and aerosolized saline without alum for 30 minutes per day on 3 days per week for 8 weeks beginning on the 21st day of the study.[18,19] Exposures were conducted

in a whole-body inhalation exposure system. A solution of 2.5% OVA in normal saline was aerosolized by delivery of compressed air to a SideStream jet nebulizer (Philips Respironics, Pittsburgh, Pennsylvania) and was injected into a chamber. The aerosol generated by this nebulizer comprised >80% particles with a diameter <4 μm. Particle concentration was maintained in the range of 10 to 20 mg/mm^3.[19]

Study Drugs

Glycyrrhizin was purchased from Sigma-Aldrich, and it was given at a dose of 10 mg/kg/d once daily for 7 consecutive days via orogastric gavage. Dexamethasone was given to the mice at a dose of 1 mg/kg/d via orogastric gavage for 7 days. The drugs and saline were given in the last week of OVA exposure for 7 consecutive days.

Histopathologic Analysis

Animals were humanely killed by an overdose of ketamine (500 mg/kg intraperitoneally) 24 hours after the last OVA and drug exposure, and histopathologic specimens were collected. A veterinary surgeon blinded to treatment group removed the tissue specimens from the middle zone of the left lung of mice, and the tissue specimens were then coded. The remaining procedures for the evaluation of the removed tissue samples were performed by 2 histopathologists blinded to treatment group. One histopathologist evaluated the findings from light microscopy, and the other conducted the electron microscopic evaluation. For electron microscopic evaluation, tissue samples of 1 to 2 mm^3 were stocked. Histopathologic samples were fixed in 10% formalin for light microscopic evaluation. After fixation, a slice from the middle zone of the left lung was embedded in paraffin. Serial sections cut at 5 μm were stained with hematoxylin-eosin (for routine histopathologic examination), with toluidine blue (for enumeration of mast cells), and with periodic acid–Schiff (for enumeration of goblet cells). Photomicrographs were taken using a JVC TK-890-E camera (JVC, Yokohama, Japan) adapted on an Olympus BH-2 RFCA microscope (Olympus Optical Co. Ltd., Tokyo, Japan). Serial sections were photographed at different magnifications by skipping over 5 fields. To evaluate the thickness of epithelium and subepithelial smooth muscle layers, measurements were taken from 4 points of each airway, and 20 measurements were taken for each mouse. Goblet cells around the airway lumina were enumerated. Periodic acid–Schiff–positive goblet cell numbers in 100 μm of tissue sections were analyzed for each airway.

Blinded histologic analysis was performed using University of Texas Health Sciences Center San Antonio ImageTool for Windows version 3.0 software (http://ddsdx.uthscsa.edu/dig/itdesc.html) after the images were transferred from a light microscope to a computer.

Samples were fixed in 2% glutaraldehyde for electron microscopic evaluation. A Libra 120 Carl Zeiss electron microscope (Carl Zeiss SMT GmbH, Oberkochen, Germany) was used for this evaluation. Photomicrographs were taken using a JVC TK-890-E camera. 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. Basal membrane thicknesses of samples of respiratory epithelium were examined using an electron microscopy program (ITEM version 5.0; Olympus Soft Imaging Solutions GmbH, Munster, Germany).

Statistical Analysis

SPSS version 11 (SPSS Inc., Chicago, Illinois) was used for statistical analysis. All the results are presented as mean (SD) from the number of experiments indicated. The normally distributed results for each outcome measure were evaluated using the Kolmogorov-Smirnov test. Because all the histopathologic parameters (the thickness of epithelium, subepithelial smooth muscle layers, and basement membrane and the number of goblet and mast cells) were normally distributed, the differences among groups were determined using 1-way ANOVA with Bonferroni correction. A $P < 0.05$ was considered statistically significant.

RESULTS

The mean (SD) basement membrane thickness was 306.34 (36.91) nm (range, 242.56–382.5 nm) in the control group, 657.52 (98.99) nm (range, 500.51–778.67 nm) in the placebo group, 405.13 (96.1) nm (range, 271.98–600.14 nm) in the dexamethasone group, and 465.01 (121.48) nm (range, 301.91–703.62 nm) in the glycyrrhizin group. The mean (SD) epithelium thickness was 19.48 (1.22) μm (range, 16.85–20.45 μm) in the control group, 41.62 (5.49) μm (range, 31.23–47.56 μm) in the placebo group, 22.59 (3.18) μm (range, 17.51–26.54 μm) in the dexamethasone group, and 25.54 (4.68) μm (range, 19.59–35.01 μm) in the glycyrrhizin group. The mean (SD) subepithelial smooth muscle thickness was 7.22 (1.37) μm (range, 4.84–9.5 μm) in the control group,11.24 (1.85) μm (range, 7.56–13.03 μm) in the placebo group, 5.62 (1.15) μm (range, 3.32–6.69 μm) in the dexamethasone group, and 7.76 (1.11) μm (range, 6.38–9.68 μm) in the glycyrrhizin group.

The mean (SD) number of mast cells in the control group was 1.34 (0.19)/16,400 μm^2 (range, 1–1.6/16,400 μm^2), in the placebo group was 3.62 (0.5)/16,400 (μm^2) (range, 2.7–4.2/16,400 μm^2), in the dexamethasone group was 2.06 (0.77)/16,400 μm^2 (range, 0.48–2.8/16,400 μm^2), and in the glycyrrhizin group was 2.77 (0.23)/16,400 μm^2 (range, 2.4–3.1/16,400 μm^2). The mean (SD) number of goblet cells was 0.32 (0.1)/100 μm (range, 0.12–0.42/100 μm) in the control group, 4.92 (0.82)/100 μm (range, 3.65–5.89/100 μm) in the placebo group, 0.66 (0.06)/100 μm (range, 0.56–0.75/100 μm) in the dexamethasone group, and 0.98 (0.15)/100 μm (range, 0.8–1.24/100 μm) in the glycyrrhizin group. The mean (SD) and range values of the histopathologic parameters for all 4 groups are demonstrated in the Table.

Compared with the control group, the asthma (placebo) group had significantly higher numbers of goblet and mast cells and increased epithelium, basement membrane, and subepithelial smooth muscle layer thickness ($P < 0.001$ for all). These results reveal that the chronic asthma model of mice was successfully established in this study.

Table. Histopathologic parameters for the control, placebo (asthmatic mice), dexamethasone, and glycyrrhizin groups. Data are given as mean (SD) [range].

Parameter	Control Group	Placebo Group	Dexamethasone Group	Glycyrrhizin Group
Basement membrane thickness, nm	306.34 (36.91)* [242.56–382.5]	657.52 (98.99) [500.51–778.67]	405.13 (96.1)* [271.98–600.14]	465.01 (121.48)*,† [301.91–703.62]
Subepithelial smooth muscle layer thickness, μm	7.22 (1.37)* [4.84–9.5]	11.24 (1.85) [7.56–13.03]	5.62 (1.15)* [3.32–6.69]	7.76 (1.11)*,† [6.38–9.68]
Epithelium thickness, μm	19.48 (1.22)* [16.85–20.45]	41.62 (5.49) [31.23–47.56]	22.59 (3.18)* [17.51–26.54]	25.54 (4.68)*,† [19.59–35.01]
Mast cell number/16,400 μm^2	1.34 (0.19)* [1–1.6]	3.62 (0.5) [2.7–4.2]	2.06 (0.77)* [0.48–2.8]	2.77 (0.23)†,‡ [2.4–3.1]
Goblet cell number/100 μm	0.32 (0.1)* [0.12–0.42]	4.92 (0.82) [3.65–5.89]	0.66 (0.06)* [0.56–0.75]	0.98 (0.15)*,† [0.8–1.24]

*$P \leq 0.001$ in groups versus placebo.

†$P > 0.05$ in the dexamethasone group versus the glycyrrhizin group.

‡$P < 0.02$ in the glycyrrhizin group versus placebo.

Evaluation of lung histopathologic findings demonstrate that the number of goblet ($P < 0.001$) and mast ($P < 0.02$) cells and the thickness of basement membrane ($P < 0.001$), subepithelial smooth muscle layers ($P \leq 0.001$), and epithelium of the lung ($P < 0.001$) were significantly lower in the glycyrrhizin group compared with the placebo group.

Comparing the glycyrrhizin and dexamethasone groups, no significant differences were found in the number of goblet ($P = 0.988$) and mast ($P = 0.075$) cells or in the thickness of basement membrane ($P = 0.514$), subepithelial smooth muscle layers ($P = 0.054$), and epithelium of the lung ($P = 1.0$). The effects of placebo, dexamethasone, and glycyrrhizin use on the histopathologic parameters and comparisons among the groups are shown in **Figure 1**.

Histopathologic views of airways are shown in **Figures 2** and **3** In the airways of asthmatic mice (placebo group), increased goblet cell numbers and thickened epithelium, basement membrane, and subepithelial smooth cell layers are seen (**Figures 2A** and **3A**). Most of the long-term histopathologic changes were ameliorated after dexamethasone administration (**Figures 2B** and **3B**). Some of the long-term changes were alleviated after glycyrrhizin administration to asthmatic mice (**Figures 2C** and **3C**).

DISCUSSION

Asthma is a chronic, immunologically mediated disease with a disturbance of the normal airway repair mechanism that results in inflammatory changes and structural alterations in the airways.[20] These structural changes include reticular basement membrane thickening due to deposition of collagen, airway smooth muscle hypertrophy/hyperplasia, goblet cell hyperplasia, and increased vascularity of the mucosa.[21–23] It seems important to prevent these structural changes (airway remodeling) in the long-term management of asthma, which prevents progression of disease severity.[23] Although current asthma therapies are effective in reducing inflammation, airway remodeling is poorly responsive to these medications.[24] Further attempts to develop new strategies to reverse airway remodeling are awaited.

Licorice (*Glycyrrhiza*) species have long been used worldwide as herbal medicines and natural sweeteners. Licorice root is accepted as a traditional medicine used mainly for the treatment of peptic ulcer, hepatitis C, and pulmonary and skin diseases.[25] This herb has several other useful pharmacologic properties, such as anti-inflammatory, antiallergic, antioxidative, antimicrobial, anticancer, immunomodulatory, hepatoprotective, and cardioprotective effects.[26] Glycyrrhizin is a triterpene glycoside obtained from licorice root (*G glabra*). The antiallergic effects of licorice are mainly due to glycyrrhizin, 18β-glycyrrhetinic acid, and liquiritigenin.[27] Ram et al[17] demonstrated that glycyrrhizin significantly reduces OVA-induced airway constriction and airway hyperreactivity to methacholine and decreases lung inflammation, including eosinophil infiltration, in the mouse model of asthma. In this study, glycyrrhizin reduced OVA-specific IgE levels in serum and T_H2 cytokine, interleukin (IL)-4, and IL-5 levels in bronchoalveolar lavage fluid, and it prevented decreases in total IgG2a levels

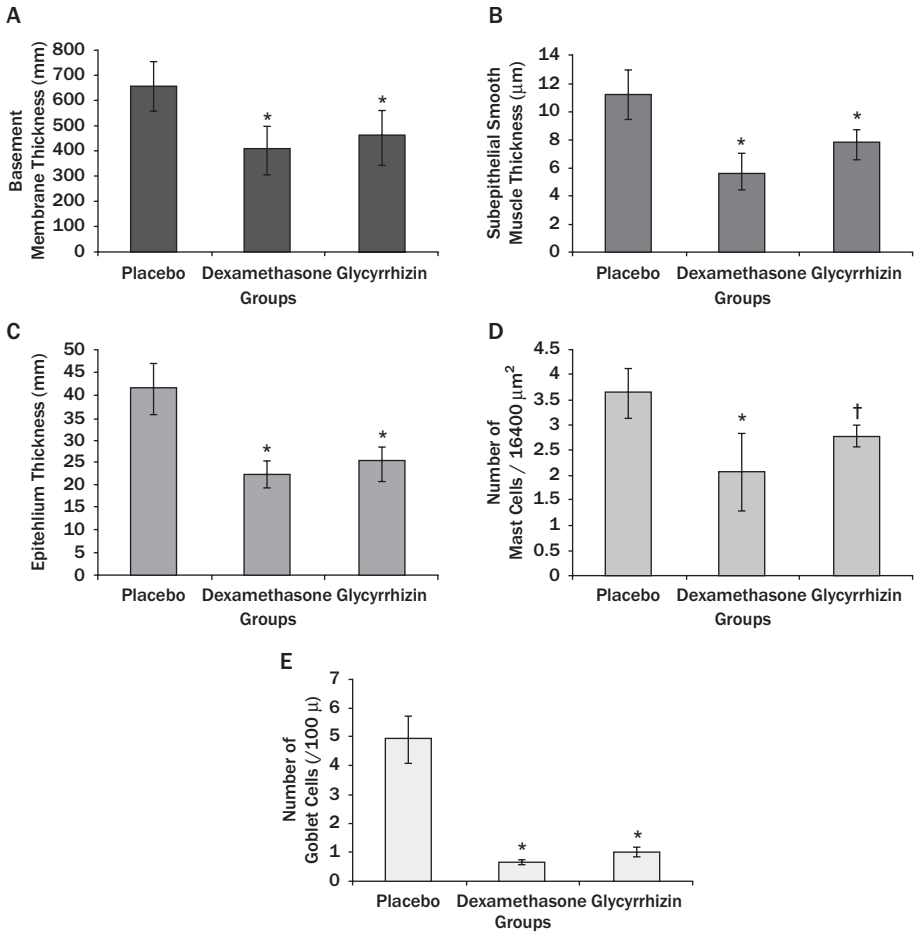

Figure 1. Effects of placebo, dexamethasone, and glycyrrhizin use on the thickness of (A) basement membrane, (B) subepithelial smooth muscle, and (C) epithelium and on the number of (D) mast cells and (E)goblet cells. Results represent the mean (SD). *$P \leq 0.001$, compared with the placebo group. †$P < 0.02$ compared with the placebo group.

in serum and T_H1 cytokine and interferon-γ levels in bronchoalveolar lavage fluid. The results of this study indicate that glycyrrhizin modulates the T_H1/T_H2 paradigm and alleviates asthmatic features in mice.[17] Glycyrrhizin has also been demonstrated to inhibit E-selectin–, L-selectin–, and P-selectin–mediated human eosinophil and neutrophil adhesion to umbilical vein endothelial cells.[28] This component of licorice root also inhibits the eotaxin-1 level via STAT6 in human lung fibroblasts[29] and nuclear factor-κB activity and IL-8 expression in cultured lung epithelial cells.[30,31] Although antiallergic and anti-inflammatory properties are reported, there is no study

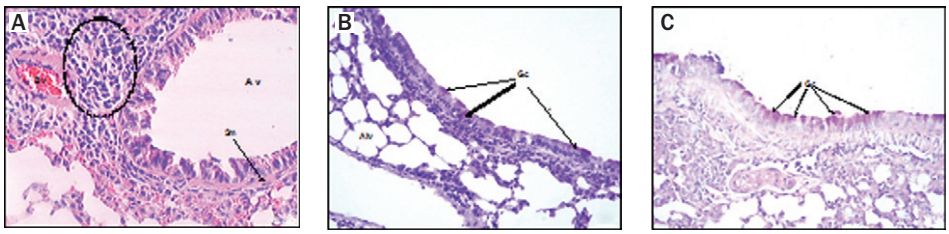

Figure 2. Light microscopic findings in the placebo, dexamethasone, and glycyrrhizin groups. (A) In the placebo (asthma) group, light microscopic findings revealed irregular respiratory epithelium. Subepithelial smooth muscle (Sm) was markedly thickened. Inflammatory cell infiltration is circled (hematoxylin-eosin). (B) In the dexamethasone group, respiratory epithelium appears regular, and the number of goblet cells (Gc) and the thickness of the smooth muscle layer are decreased (periodic acid–Schiff, ×20). (C) In the glycyrrhizin group, airway epithelium and parenchymal areas appear normal. The number of Gc is decreased (periodic acid–Schiff, ×20).

reported in the English literature, to our knowledge, evaluating the effect of glycyrrhizin on airway remodeling. In the present study, we showed that mice receiving glycyrrhizin had alleviation of all the evaluated established long-term histopathologic changes of lung in the mouse model of asthma, including thickness of basement membrane, epithelium, and smooth muscle layer of airways and number of goblet and mast cells.

Dexamethasone is accepted as a potent inhibitor of airway inflammation and remodeling.[32] Administration of corticosteroids has been shown to inhibit the struc-

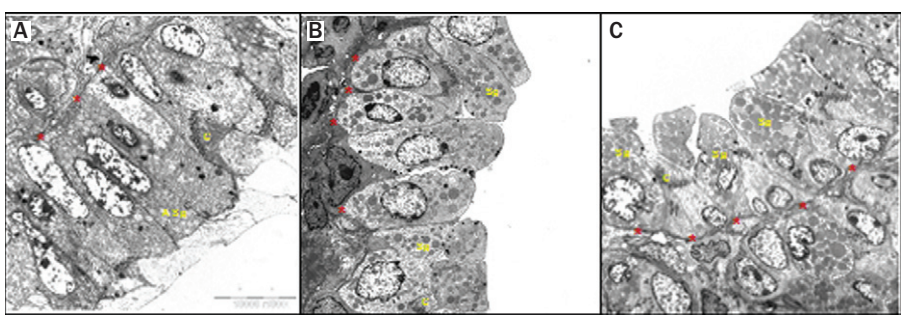

Figure 3. Electron microscopic findings in the placebo, dexamethasone, and glycyrrhizin groups. (A) In the asthma group, basement membrane (*) integrity was disrupted. Cells filled with secretion granules were detected in the electron microscopic evaluation. (B) In the dexamethasone group, basement membrane (*) was regular. Secretory cells were filled with secretion granules. (C) In the glycyrrhizin group, basement membrane (*) was regular, and cells with cilia and secretory cells were filled with dense secretion granules and were seen as normal.

tural changes associated with airway fibrosis in other animal models.[33] Although treatment with dexamethasone, one of the most potent corticosteroids, improved all the histopathologic parameters in the present study, no significant differences were found in the histopathologic parameters between the glycyrrhizin and dexamethasone groups. Although not to a statistically significant degree, it was found that mice given dexamethasone had decreased smooth muscle thickness and fewer goblet cells compared with those receiving glycyrrhizin. The similiar effectiveness of dexamathasone and glycyrrhizin on long-term structural changes in asthmatic airways in the present study may be due to the glucocorticoid-like inhibitory effect of glycyrrhizin in lung cells.[30] Although the results of this study show that asthmatic mice given glycyrrhizin had a benefit regarding long-term structural changes in the airways, these data are not yet enough to claim that glycyrrhizin could be an alternative to dexamethasone in long-term asthma treatment. More long-term studies are needed to show the efficacy of glycyrrhizin in the treatment of chronic asthma.

There are some important limitations of this study: cytokine levels, which have an important role in asthma pathogenesis, could not be evaluated; a small number of animals was used (possibility that type I and II errors exist); and the results may not directly translate to positive findings in human clinical trials.

CONCLUSIONS

Glycyrrhizin had a beneficial effect in treating all the evaluated established long-term histopathologic changes of lung in the mouse model of asthma. Glycyrrhizin may be a promising alternative asthma treatment. Further studies are needed to evaluate the effects of glycyrrhizin on asthmatic airways.

ACKNOWLEDGMENTS

All authors contributed to designing the study, collecting and analyzing the data, writing, and revising the manuscript.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.

REFERENCES

1. Janson C. The importance of airway remodelling in the natural course of asthma. *Clin Respir J*. 2010;4(Suppl 1):28–34.
2. Sumi Y, Hamid Q. Airway remodeling in asthma. *Allergol Int*. 2007;56:341–348.
3. Kips JC, Pauwels RA. Airway wall remodelling: does it occur and what does it mean? *Clin Exp Allergy*. 1999;29:1457–1466.
4. Childhood Asthma Management Program Research Group. Long-term effects of budesonide or nedocromil in children with asthma. *N Engl J Med*. 2000;343:1054–1063.
5. Skoner JD, Schaffner TJ, Schad CA, et al. Addressing steroid phobia: improving the risk-benefit ratio with new agents. *Allergy Asthma Proc*. 2008;29:358–364.
6. Li XM. Complementary and alternative medicine in pediatric allergic disorders. *Curr Opin Allergy Clin Immunol*. 2009;9:161–167.

7. Sharafkhaneh A, Velamuri S, Badmaev V, et al. The potential role of natural agents in treatment of airway inflammation. *Ther Adv Respir Dis*. 2007;1:105–120.
8. Ziment I. Recent advances in alternative therapies. *Curr Opin Pulm Med*. 2000;6:71–78.
9. Pinn G. Herbal therapy in respiratory disease. *Aust Fam Physician*. 2001;30:775–779.
10. Huntley A, Ernst E. Herbal medicines for asthma: a systematic review. *Thorax*. 2000;55:925–929.
11. Litvinenkoand VI, Obolentseva GV. Chemical and pharmaceutical research on flavanoids of *Glycyrrhiza glabra* L.and *Glycyrrhiza uralensis* Fisch. *Med Prom SSSR*. 1964;18:20–23.
12. Matsui S, Matsumoto H, Sonoda Y, et al. Glycyrrhizin and related compounds down-regulate production of inflammatory chemokines IL-8 and eotaxin 1 in a human lung fibroblast cell line. *Int Immunopharmacol*. 2004;4:1633–1644.
13. Zhong Z, Wen Z, Darnell JE Jr. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science*. 1994;264: 95–98.
14. Menegazzi M, Di Paola R, Mazzon E, et al. Glycyrrhizin attenuates the development of carrageenan-induced lung injury in mice. *Pharmacol Res*. 2008;58:22–31.
15. Tamaya T, Sato S, Okada HH. Possible mechanism of steroid action of the plant herb extracts glycyrrhizin, glycyrrhetinic acid, and paeoniflorin: inhibition by plant herb extracts of steroid protein binding in the rabbit. *Am J Obstet Gynecol*. 1986;155:1134–1139.
16. Krahenbuhl S, Hasler F, Frey BM, et al. Kinetics and dynamics of orally administered 18 β-glycyrrhetinic acid in humans. *J Clin Endocrinol Metab*. 1994;78:581–585.
17. Ram A, Mabalirajan U, Das M, et al. Glycyrrhizin alleviates experimental allergic asthma in mice. *Int Immunopharmacol*. 2006;6:1468–1477.
18. Temelkovski J, Hogan SP, Shepherd DP, et al. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax*. 1998;53:849–856.
19. Babayigit A, Olmez D, Karaman O, et al. Effects of ginkgo biloba on airway histology in a mouse model of chronic asthma. *Allergy Asthma Proc*. 2009,30:186-191.
20. Murphy DM, O'Byrne PM. Recent advances in the pathophysiology of asthma. *Chest*. 2010; 137:1417–1426.
21. Kips JC, Pauwels RA. Airway remodelling: does it occur and what does it mean? *Clin Exp Allergy*. 1999;2:1457–1466.
22. Lloyd CM, Robinson DS. Allergen-induced airway remodeling. *Eur Respir J*. 2007;29:1020–1032.
23. Tagaya E, Tamaoki J. Mechanisms of airway remodeling in asthma. *Allergol Int*. 2007;56: 331–340.
24. Payne DN, Rogers AV, Adelroth E, et al. Early thickening of the reticular basement in children with difficult asthma. *Am J Respir Crit Care Med*. 2003;167:78–82.
25. Asl MN, Hosseinzadeh H. Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. *Phytother Res*. 2008;22:709–724.
26. Ross IA. *Medicinal Plants of the World.* Totowa, NJ: Humana Press Inc; 2001.
27. Shin YW, Bae EA, Lee B, et al. In vitro and in vivo antiallergic effects of *Glycyrrhiza glabra* and its components. *Planta Med*. 2007;73:257–261.
28. Kim MK, Brandley BK, Anderson MB, Bochner BS. Antagonism of selectin-dependent adhesion of human eosinophils and neutrophils by glycomimetics and oligosaccharide compounds. *Am J Respir Cell Mol Biol*. 1998;19:836–841.
29. Matsui S, Sonoda Y, Sekiya T, et al. Glycyrrhizin derivative inhibits eotaxin 1 production via STAT6 in human lung fibroblasts. *Int Immunopharmacol*. 2006;6:369–375.

30. Takei H, Baba Y, Hisatsune A, et al. Glycyrrhizin inhibits interleukin-8 production and nuclear factor-κB activity in lung epithelial cells, but not through glucocorticoid receptors. *J Pharmacol Sci*. 2008;106:460–468.
31. Iino S, Tango T, Matsushima T, et al. Therapeutic effects of stronger neo-minophagen C at different doses on chronic hepatitis and liver cirrhosis. *Hepatol Res*. 2001;19:31–40.
32. Blyth DI, Wharton TF, Pedrick MS, et al. Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-interleukin-5 antibody. *Am J Respir Cell Mol Biol*. 2000;23:241–246.
33. Trifilieff A, El-Hashim A, Bertrand C. Time course of inflammatory and remodeling events in a murine model of asthma: effect of steroid treatment. *Am J Physiol Lung Cell Mol Physiol*. 2000;279:1120–1128.

Address correspondence to: Arzu Babayigit Hocaoglu, MD, Extend, Memduh Asaf sok., No:1, (Ledra Palace isiklari yani), Lefkosa, North Cyprus. E-mail: arbabayigit@yahoo.com