

Preliminary report

Efficacy of a traditional Korean medicine, Chung-Sang-Bo-Ha-Tang, in a murine model of chronic asthma

Gu Seob Roh^a, Seong-Won Seo^a, SinKoo Yeo^a, Jung Min Lee^a, Jung-Won Choi^a,
EunKyung Kim^a, YoungHyun Shin^a, Chongwoon Cho^b, Hyunsu Bae^c,
Sung-Ki Jung^d, KyuBum Kwack^{a,*}

^aNational Genome Research Institute, National Institute of Health, 5 Nokbun-dong, Eunpyung-gu, Seoul 122-701, South Korea

^bPurimed R and D Institute, College of Oriental Medicine, Kyung Hee University, Hoegi-dong, Dongdaemoon-ku, Seoul 130-702, South Korea

^cDepartment of Physiology, College of Oriental Medicine, Kyung Hee University, Hoegi-dong, Dongdaemoon-ku, Seoul 130-702, South Korea

^dDepartment of Internal Medicine, College of Oriental Medicine, Kyung Hee University, Hoegi-dong, Dongdaemoon-ku, Seoul 130-702, South Korea

Received 3 May 2004; received in revised form 2 June 2004; accepted 24 September 2004

Abstract

Traditional herbal medicines may be viable alternatives to corticosteroid therapy for treatment of asthma. However, the therapeutic mechanisms of herbal compounds remain a matter of considerable debate. This study was performed to evaluate the effects of Chung-Sang-Bo-Ha-Tang (CSBHT), a herbal compound administrated therapeutically to asthma patients for centuries, on airway inflammation and remodeling in a murine model of chronic asthma. BALB/c mice sensitized to ovalbumin (OVA) were chronically challenged with aerosolized OVA for 6 weeks. During the last 2 weeks, some mice were treated daily with CSBHT by intragastric feeding. Dexamethasone (Dex)-treated, phosphate-buffered saline (PBS)-treated, and naïve mice served as controls. The effects of CSBHT on airway inflammation, lung pathology, and cytokine production were evaluated. Mice exposed to recurrent airway challenge with OVA had chronic inflammation and characteristics of airway remodeling, including subepithelial fibrosis, epithelial hypertrophy, and goblet cell hyperplasia. CSBHT was as effective as Dex at moderately reducing these changes compared to the PBS-treated mice. In addition, IL-5 and IFN- γ levels in supernatants of Concanavalin A (Con A)-activated splenocyte cultures were reduced in mice treated with CSBHT. Treatment with CSBHT during the last 2 weeks of challenge modulated airway inflammation and remodeling in a murine model of chronic asthma. Thus, CSBHT may effectively delay the progression of airway inflammation and remodeling.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Chung-Sang-Bo-Ha-Tang; Chronic asthma; Mice

Abbreviations: CSBHT, Chung-Sang-Bo-Ha-Tang; OVA, ovalbumin; Con A, concanavalin A; Dex, dexamethasone; PBS, phosphate-buffered saline; CAM, complementary or alternative medicine; H&E, hematoxylin and eosin; PAS, periodic acid-Schiff; MT, Masson trichrome.

* Corresponding author. Tel.: +82 2 380 1523; fax: +82 2 354 1063.

E-mail addresses: kkwack@nih.go.kr; kkwack@chamc.co.kr (K.B. Kwack).

1567-5769/\$ - see front matter © 2004 Elsevier B.V. All rights reserved.

doi:10.1016/j.intimp.2004.09.036

1. Introduction

Asthma is a chronic inflammatory disease characterized by airway inflammation, increased mucus production, intermittent airway obstruction and hyper-responsiveness [1,2]. Airway remodeling, in particular, is characterized by structural and morphometric changes including subepithelial fibrosis, epithelial hypertrophy, goblet cell hyperplasia, and smooth muscle hypertrophy [3,4]. Over the last decade, investigations into the pathogenesis of chronic asthma have focused on the Th1/Th2 paradigm in asthmatics and animal models [5], but the pathologic mechanisms have not yet been completely elucidated.

Anti-inflammatory therapy, the established asthma treatment during the recent past decades, has been shown to have no effect on airway remodeling [6]. Patients with chronic asthma who take these drugs for a long and indefinite period of time may develop serious side effects, particularly over the long term [7]. Consequently, new or alternative approaches to modulate this disease are badly needed. Complementary or alternative medicines (CAMs) have become an increasingly appealing component of standard medical care in Western countries. Asthma is one of a number of common diseases for which CAM therapies are useful. Ernst [8] reported that 80% of patients with asthma and other diseases are satisfied with their experiences using CAM. Chung-Sang-Bo-Ha-Tang (CSBHT) has been used to treat chronic asthma in Korea for centuries. Previous clinical findings indicated that CSBHT improved the Quality of Life Questionnaire for Adult Korean Asthmatics scores, increased forced expiratory volumes_{1.0} (FEV_{1.0}) and peak expiratory flow rates (PEFR), and reduced the need for steroids and β_2 -agonists [9]. However, the therapeutic mechanisms of this CSBHT are still far from clear.

Therefore, we compared the effects of CSBHT and dexamethasone (Dex) on airway inflammation and remodeling in a murine model of chronic asthma.

2. Materials and methods

2.1. Mice

Female BALB/c mice (5 weeks old) purchased from Japan SLC (Hamamatsu, Japan) were maintained in

the animal facility at the National Genome Research Institute of Korea National Institute of Health. Mice were treated in accordance with standard guidelines for laboratory animal care [10].

All mice were provided water and standard chow ad libitum for 1 week prior to any experimental procedures to allow them to acclimatize to their new surroundings.

2.2. Preparation of CSBHT extract

CSBHT, contains 18 species of medicinal plants, was purchased from Korean Association of Crude Medicinal Herbs and washed by using third distilled water. Every herb of CSBHT was ground to fine powder and mixed with amount of 1180 g as the ratio indicated in Table 1. It was extracted with 70% ethanol using a sonicator (Branson, USA) for 10 min. The precipitate was successively re-extracted with 80%, 90% and 100% ethanol, and the whole supernatant was concentrated at 60 °C under vacuum using an evaporative system (Eyela, Japan) and freeze-dried in a freeze dryer (Eyela); 39.70 g (yield: 3.36%) of extract powder was obtained. The sample was dissolved in phosphate-buffered saline (PBS) and sterilized by passing through 0.22 μ m syringe filter.

2.3. The HPLC analysis of standard materials to test samples

The dried ethanol extract, about 240 mg of CSBHT was accurately weighed, and then put in test tube, and dissolved in 4 ml of 50% methanol (HPLC reagent, J.T. Baker, USA) followed by filtering using 0.45 μ m syringe filter (PVDF, Waters, Milford, USA). Each marker substances (standard materials) used for the quantitative analysis to CSBHT was purchased; 5-HMF (Aldrich Chemicals, USA), allantoin (Aldrich Chemicals), loganin (Wako, Japan), paeonol (Wako), poncirin (Fluka Chemie, Germany), berberine (Sigma, Germany), baicalin (Aldrich Chemicals), homogentisic acid (Fluka Chemie), glycyrrhizic acid (Sigma, Germany), and schizandrin (Wako). The standard materials were weighted 10 mg, and were dissolved according to the analysis condition of standard materials. The dissolved standard solution was diluted as 0.1, 0.5, 1.0, 1.5, 2.0 mg/ml, respectively, and the

standard HPLC chromatogram was then obtained. The relationship between the concentration and the peak-area was measured by minimum square method (R^2 value). The HPLC apparatus was a Waters Breeze System (717+Autosampler, 2487 dual λ absorbance detector, 1525 binary HPLC Pump, Waters), and Waters Breeze System (Ver. 3.20, Waters) was used for data acquisition and integration.

The quantity of standard materials contained in CSBHT were calculated by the following formula:

The amount (mg) of standard materials

$$= \{ \text{the quantitative amount (mg) of standard materials} \times \text{AT/AS} \} / n \quad (n = 3)$$

(cf., AT: the peak-area of test samples containing standard materials, AS: the peak-area of standard materials).

The standard materials for quantitative analysis of each herb medicines containing CSBHT were shown in Table 1. From the result of standard calibration curve, R^2 values of all marker substances are between 0.991 and 0.999. The amounts of standard materials in CSBHT are indicated in Table 1.

The dose of 100 μ l per mouse daily for 2 weeks used in this study was based on a conversion table of equivalent effective dose ratios in humans and animals based on body surface area [11].

2.4. Antigen sensitization, challenge, and treatment

Mice were sensitized on days 0 and 6 of the experiment by two intraperitoneal injections of 20 μ g chicken egg ovalbumin (OVA, grade V, Sigma, St. Louis, MO, USA) emulsified in 1 mg of aluminum hydroxide (Pierce Chemical, Rockford, IL, USA) in a total volume of 200 μ l. Two weeks after the last sensitization, mice were exposed to 1% OVA aerosolized for 30 min/day, 3 days/week for 6 weeks. OVA challenge was performed by placing mice in a Plexiglas box (29 $\times$ 22 $\times$ 18 cm) and aerosolizing OVA using an ultrasonic nebulizer (NE-U12; Omron, Tokyo, Japan) driven by compressed air for 30 min. During the last 2 weeks, CSBHT (1000 mg/kg) was intragastrically administered daily for 14 consecutive days. Intragastric feeding was performed with a 25-gauge stainless steel blunt feeding needle (Fine Science Tool, Foster, CA). Dex (Sigma, St. Louis,

Table 1

Contents of Chung-Sang-Bo-Ha-Tang (CSBHT) and the amounts of standard materials

Contents	Quantity (%)	Standard materials (SM)	SM quantities (mg/CSBHT, ex. 1 g)
Rehmannia Radix vaporata	6.78	5-HMF	0.27 $\pm$ 0.029
Discorea Radix	6.78	Allantoin	3.14 $\pm$ 0.402
Corni Fructus	6.78	Loganin	5.01 $\pm$ 0.038
Hoelen	6.78		
Mountain Cortex Radicis	6.78	Paeonol	0.83 $\pm$ 0.077
Alismatis Radix	6.78		
Maximowicziae Fructus	5.08	Schizandrin	0.90 $\pm$ 0.353
Ponciri Fructus	5.08	Poncirin	24.03 $\pm$ 0.05
Liriois Tuber	5.08		
Asparagi Radix	5.08		
Fritillariae roylei Bulbus	5.08		
Platycodi Radix	5.08		
Coptidis Rhizoma	5.08	Berberine	4.68 $\pm$ 0.019
Ansu Semen	5.08		
Pinelliae Rhizoma	5.08	Homogentisic acid	57.66 $\pm$ 6.24
Tricosanthis Semen	5.08		
Scutellariae Radix	5.08	Baicalin	8.03 $\pm$ 0.033
Glycyrrhizae Radix	3.39	Glycyrrhizic acid	2.98 $\pm$ 0.019

MO, USA)-treated mice were administered daily intraperitoneal injections of 1 mg/kg. Sham-treated mice were intragastrically administered PBS daily. Naïve mice served as additional controls. Experimental groups were sacrificed 48 h after the last exposure.

2.5. Blood

Mice were anesthetized by intraperitoneal administration of pentobarbital sodium (100 mg/kg) 48 h after the last challenge and then bled by retro-orbital puncture using heparinised capillary tubes. One drop was smeared on a slide and stained with Diff-Quik (Fisher, VA, USA) for determination of differential cell counts. The remaining portions of the blood samples were centrifuged at 540 $\times$ g for 10 min at 4 °C, and then plasma was stored at –70 °C until used.

2.6. Bronchoalveolar lavage fluid (BALF)

After removal of blood, mice were used either for BALF ($n=4-5$). The BAL was performed for differential cell counters and measurement of cytokines. The tracheas of mice were cannulated and lavaged with two 0.8 ml aliquots of PBS. The BALF was centrifuged and supernatants were collected and stored at -70°C until used. The total number of BALF cells was counted with the use of a hemocytometer. Differential BALF cell counts were determined by microscopic evaluation of 300 cells per Diff-Quik-stained cytospin slide.

2.7. Measurement of total IgE in plasma

Plasma samples from each experimental group were analyzed for total IgE. Complementary capture and detection antibody pairs for mouse IgE antibody were purchased from PharMingen (San Diego, CA), and the IgE enzyme-linked immunosorbent assay (ELISA) was performed according to the manufacturer's directions. Duplicate plasma samples were diluted 1:100. IgE levels in each sample were measured from optical density readings at 450 nm, and IgE concentrations were calculated from a standard curve generated using recombinant IgE (5–2000 pg/ml).

2.8. Histologic examination and morphometry

After removal of blood, lungs were slowly perfused with 10 ml PBS via the right ventricle, and then perfused with 4% paraformaldehyde and immersed in fixative solution overnight. Lungs were embedded in paraffin, and 5 μm sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Masson Trichrome (MT). The slides were evaluated by microscopy.

Inflammation was scored in a double-blind screen with two independent investigators. The degree of peribronchial and perivascular inflammation was evaluated by a subjective scale of 0–3, as described elsewhere [12]. A value of 0 was assigned when no inflammation was detectable, a value of 1 indicated occasional cuffing with inflammatory cells, a value of 2 indicated that most bronchi or vessels were surrounded by a thin layer (one to five cells) of inflammatory cells and a value of 3 indicated that most bronchi or vessels

were surrounded by a thick layer (more than five cells) of inflammatory cells. Total lung inflammation was defined as the average of the peribronchial and perivascular inflammation scores. Section analysis for PAS staining was also performed in a blind fashion using a quantitative scoring system (0–4), where 0=no epithelial staining, 1=slight epithelial staining, 2=moderate epithelial staining, 3=heavy epithelial staining, and 4=massive epithelial staining. MT-stained sections were assessed for subepithelial fibrosis. For quantitative or semiquantitative analysis, images of these sections were examined using a Leica Qwin image analysis system (Leica, Cambridge, United Kingdom) calibrated with a reference measurement slide. The mean thickness of subepithelial fibrosis for each animal was calculated.

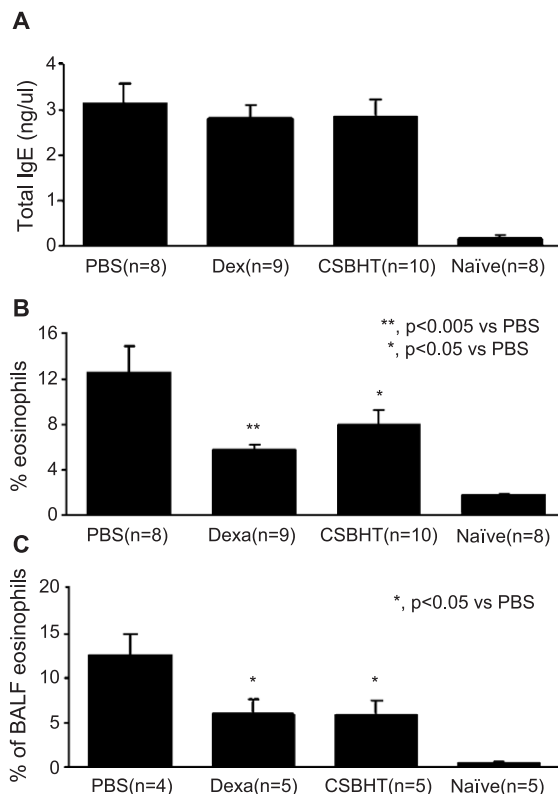


Fig. 1. Effect of CSBHT on total plasma IgE and the percentage of eosinophils in peripheral blood and BALF. After 48 h after the last challenge, mice were killed and lungs lavaged. Total plasma IgE was measured using ELISA (A). Peripheral blood was smeared on a slide and stained with Diff-Quik to determine differential cell counts (B). Differential BALF cell counts were also determined on Diff-Quik-stained cytospin slide (C). Data are represented as mean $\pm$ S.E.M. from 4 to 10 individual mice in each group.

2.9. Cell culture and cytokine assay

Spleen cells from each mouse were isolated and suspended in complete culture medium (RPMI 1640 containing 10% FBS, 1% penicillin-streptomycin, and 1% glutamate). Cells (2×10^6 /ml/well) were cultured in plates in the presence of Concanavalin A (Con A, 2 μ g/ml). Supernatants were collected after a 72-h culture period. IL-4, IL-5, and IFN- γ concentrations in spleen culture supernatants were determined by

ELISA according to the manufacturer's instructions (PharMingen).

2.10. Statistical analysis

Values are expressed as the mean $\pm$ S.E.M. Differences between the groups (PBS, Dex, and CSBHT) were determined by ANOVA, followed by Bonferoni's method for all pairwise multiple comparisons. A P value <0.05 was considered statistically significant.

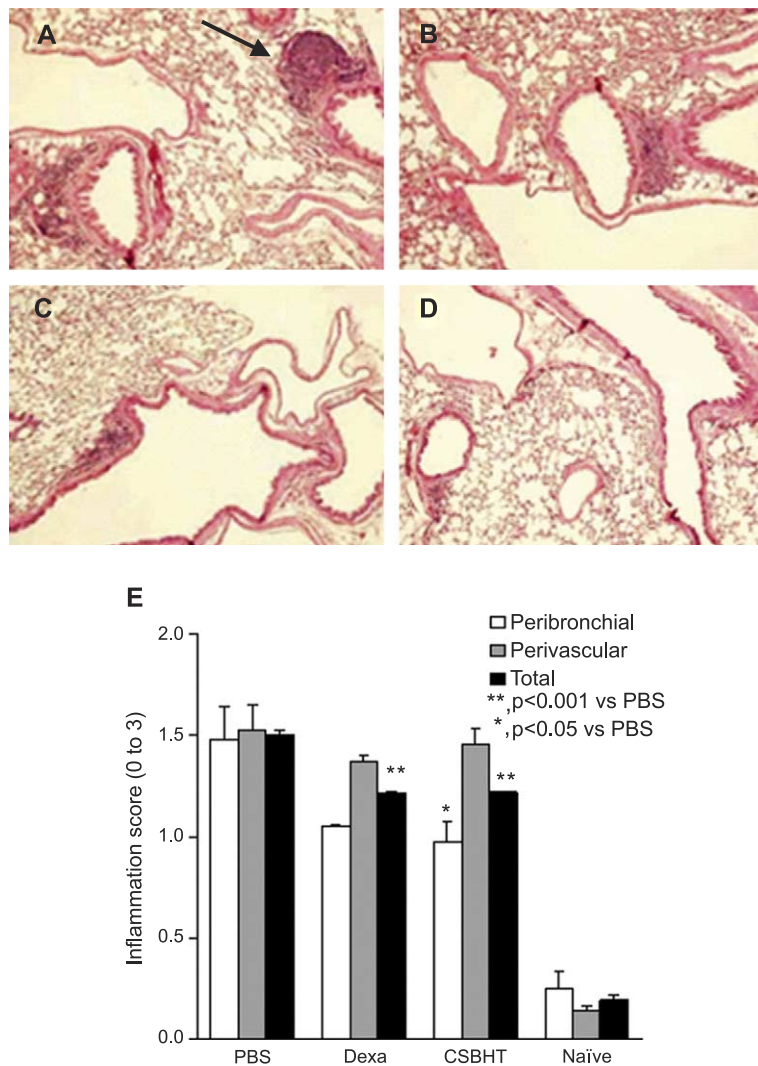


Fig. 2. Effect of CSBHT on airway inflammation. Representative H&E-stained lung sections from (A) PBS, (B) Dex, (C) CSBHT-treated mice, and (D) naïve mice. Inflammation scores are represented as mean $\pm$ S.E.M. from seven to eight mice in each group (E). Objective lens $\times 100$. Arrow indicates areas of inflammation.

3. Results

3.1. Effect of CSBHT on total plasma IgE and percentage of eosinophils in peripheral blood and BALF

Levels of total IgE were determined by ELISA in each experimental group 48 h after the last challenge. Total plasma IgE levels in all the OVA-sensitized/challenged mice were significantly higher than levels in naïve mice (Fig. 1A). Total plasma IgE levels did not differ significantly among any of the OVA-

sensitized/challenged mice. In addition, total plasma IgE levels in the Dex- or CSBHT-treated mice did not differ significantly. These results demonstrate that CSBHT, like Dex, has no effect on plasma IgE concentrations in chronic asthma resulting from chronic allergen exposure.

We measured the percentages of eosinophils in peripheral blood and BALF. Both percentages of eosinophils in all OVA-sensitized/challenged mice were significantly higher than those in the naïve mice (Fig. 1B and C). In contrast to the total IgE levels, the percentages of eosinophils in the drug-treated mice

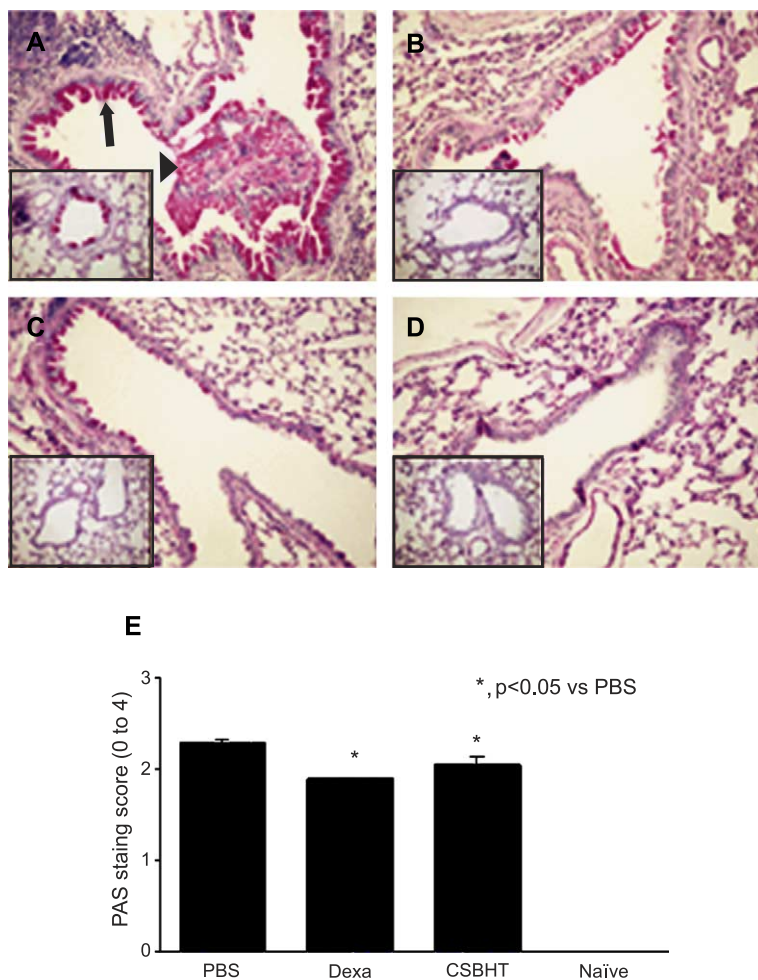


Fig. 3. Effect of CSBHT on mucus hyperproduction. Representative PAS-stained lung sections from (A) PBS, (B) Dex, (C) CSBHT-treated mice, and (D) naïve mice. PAS staining scores are represented as mean $\pm$ S.E.M. from seven to eight mice in each group (E). Objective lens $\times 200$ for low-power field and $\times 400$ for high-power field inserts. Arrow indicates intense pink staining illustrating mucus within goblet cells of the respiratory epithelium. Arrowhead indicates mucus plug. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were significantly lower than the percentage of eosinophils in PBS-treated mice. The percentages of eosinophils did not differ significantly between CSBHT- and Dex-treated mice, even though the anti-eosinophilic effect of Dex appeared to be greater than that of CSBHT in peripheral blood.

3.2. Effect of CSBHT on lung histology

CSBHT or Dex treatment did not induce any observed side effects in the experimental groups studied. The effects of CSBHT on airway inflammation and remodeling had not previously been studied in a murine model of chronic asthma. We evaluated the histologic effects of CSBHT on airway inflammation and remodeling with H&E, PAS, and MT stains.

To evaluate airway inflammation, we stained lung sections with H&E and calculated inflammation scores as described in Materials and methods. We found marked peribronchial and perivascular inflammatory infiltrates in lung sections of OVA-sensitized/challenged mice compared with naïve mice (Fig. 2A–D). The scores of peribronchial, perivascular, and total lung inflammation were significantly higher in PBS-treated mice than in naïve mice (Fig. 2E). The increased peribronchial and total lung inflammation scores were moderately reduced by treatment with CSBHT or Dex, but not perivascular inflammation. These results demonstrate that CSBHT, like Dex, inhibits OVA-induced inflammation in the lungs.

Airway remodeling is characterized by excessive production and hypersecretion of viscous mucus. To evaluate for goblet cell hyperplasia, we stained lung sections with PAS. PAS-stained goblet cells, which were virtually absent in the airways of the naïve mice, were clearly presented in all the OVA-sensitized/challenged mice (Fig. 3A–D). PAS staining scores were calculated for each experimental group as described in Materials and methods. PBS-treated mice had a staining score of 2.30 ± 0.02 , while Dex- and CSBHT-treated mice had a staining score of 1.89 ± 0.01 and 2.03 ± 0.01 , respectively (Fig. 3E). These results demonstrate that treatment with CSBHT or Dex influences the number of goblet cells in the airways. In particular, greater numbers of PAS-stained goblet cells were reduced in the smaller airways by CSBHT or Dex treatment (Fig. 3A–D; inserts). In

addition, mucus plugs were present particularly in the larger airways of PBS-treated mice (Fig. 3A). Fewer mucus plugs were seen in the larger airways of CSBHT- or Dex-treated mice (Fig. 3B and C). These results demonstrate that CSBHT prevents mucus production in airways.

To evaluate the effects of CSBHT on structural and morphometric changes, we stained lung sections with MT. We found marked epithelial hypertrophy and subepithelial fibrosis in PBS-treated mice compared with naïve mice (not shown). The extent of collagen infiltration in either Dex- and CSBHT-treated mice was as great as in the airways of PBS-treated mice.

3.3. Effect of CSBHT on T-cell cytokine responses

To determine a possible effect of CSBHT on T-cell responses, we evaluated the effects of CSBHT on T-cell

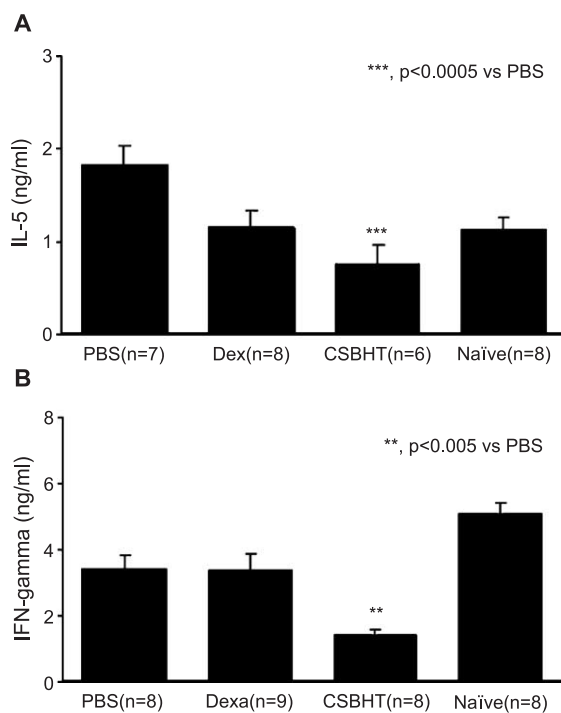


Fig. 4. Cytokine production in spleen culture supernatants. Individual spleen cells from each group were isolated and cultured in medium containing Con A ($2 \mu\text{g/ml}$). Supernatants were collected after 72 h of culture. IL-5 levels (A) and IFN- γ levels (B) were determined by ELISA. Data are represented as mean $\pm$ S.E.M. from six to nine individual mice in each group.

cytokine secretion in supernatants of Con A-activated splenocyte cultures. We measured Con A-activated IL-4, IL-5, and IFN- γ levels in supernatants of splenocyte cultures. However, IL-4 secretion levels were undetectable, so relative IL-4 concentrations (ng/ml) could not be presented. IL-5 levels were increased and IFN- γ levels were decreased in supernatants of splenocyte cultures from PBS-treated mice compared with naïve mice (Fig. 4). The increase in IL-5 levels was decreased by CSBHT treatment (Fig. 4A). Furthermore, IFN- γ levels in CSBHT-treated mice were markedly lower than levels in naïve mice (Fig. 4B).

4. Discussion

Several studies have demonstrated that certain traditional herbal medicine formulas have therapeutic benefits in allergic asthma [13,14]. On the use of this murine model for chronic asthma, Temelkovski et al. [15] reported that changes in airway remodeling in the murine model for chronic asthma used in this study, characterized by epithelial hypertrophy, subepithelial fibrosis, and goblet cell hyperplasia, develop 4 to 6 weeks after allergen exposure. Therefore, we assessed whether CSBHT inhibits progression of or reverses these changes when administered during weeks 5 and 6 of allergen exposure. The present study is the first demonstration that CSBHT has the inhibitory effects on airway inflammation and remodeling in a murine model of chronic asthma. This effect was equivalent to the effects of Dex. In particular, IL-5 and IFN- γ levels in supernatants of Con A-activated splenocyte cultures were reduced in mice treated with CSBHT. Thus, CSBHT may be a useful CAM for asthmatic patients.

Elevated serum IgE levels and peripheral eosinophilia may correlate with the incidence or severity of asthma [16]. However, this correlation is not uniform and true for all asthmatic patients. The degree of suppression of total IgE in plasma by Dex or CSBHT treatment was somewhat inconsistent with the other findings. In acute and chronic animal models, repeated OVA challenges induced a further significant increase in total serum IgE that started 1 day following the last challenge and peaked at 3 days [17]. In this study, total plasma IgE levels, 48 h after the last challenge, did not differ significantly among

the OVA-sensitized/challenged mice. This result suggests that CSBHT, like Dex, has no effect on the total IgE concentration changes that occur during the development of chronic asthma. In contrast, percentages of eosinophils in peripheral blood and BALF were decreased by drug treatment. Dex suppresses allergen-induced both IL-5 and eotaxin expression, as well as eosinophilia in animals [18,19]. In the present study, we demonstrated that CSBHT reduces the level of IL-5 in supernatants of Con A-activated splenocyte cultures, as well as inhibits eosinophilia in blood and BALF.

In particular, we examined structural changes at the level of the conducting airways. Peribronchial, perivascular, and total inflammation were severe. Treatment with CSBHT or Dex moderately inhibited airway inflammation compared with PBS-treated mice. Dex is a potent inhibitor of airway inflammation and remodeling [20]. Inhibition by these inhaled corticosteroids is well-documented in human asthma, and administration of corticosteroids has also been shown to inhibit the structural changes associated with airway fibrosis in other animal models [21].

We demonstrated that goblet cell hyperplasia was quantitatively reduced by CSBHT or Dex. In particular, large percentages of goblet cells in the distal conducting airways appeared to be dramatically reduced by CSBHT or Dex treatment. In addition, mucus plugs were significantly reduced in the airways of CSBHT-treated mice. These results suggest that CSBHT protects against mucus production. Goblet cells, normally absent in small airways [22], are observed in chronic airway inflammatory conditions and contribute to bronchiolar obstruction [23]. We suggest that the reduction in PAS-stained goblet cells in the smaller airways observed in this study may play an important role in the relief of asthmatic symptoms. However, the contribution of the airway remodeling response to the severity of asthma remains controversial because increased goblet cell numbers and collagen deposition around the airways do not appear to reflect the severity of this disease [24,25].

Recently, Romagnani [26] reported that Th2 cytokines play a central role in the pathogenesis of asthma. Since Th1 and Th2 types of reactions appear to be reciprocally regulated in vivo, modulation of the Th1/Th2 balance should become a common strategy for asthma therapy [27]. In the present study, we

found that the elevation of IL-5 levels in the supernatants of Con A-activated splenocyte cultures was decreased by CSBHT or Dex treatment. However, only detectable level of IL-5 was determined in the supernatants of Con A-activated splenocyte cultures, but not medium or OVA-activated cultures (data not shown). This is most likely due to an increase in the relative proportions of memory (having been previously exposed to OVA) vs. naïve (no previous antigenic exposure) T-lymphocytes. The decreased IL-5 levels correlated with the percentages of eosinophils in peripheral blood and BALF. These results suggest that the inhibitory effect of CSBHT on IL-5 production is associated with down-regulation of eosinophils. IL-5 mRNA expression in activated T cells is increased in lung tissue [28], and IL-5 protein levels are elevated in the serum and BALF of asthmatic patients [29]. Other studies have demonstrated that anti-IL-5 treatment completely abrogates eosinophilic airway infiltration and prevents development of airway hyperresponsiveness [30].

The Th2 response is inhibited by IFN- γ -producing Th1 cells. Low dose IFN- γ therapy had in vivo anti-inflammatory effects in an animal model [31]. However, our results demonstrated that IFN- γ levels in the supernatants of Con A-activated splenocyte cultures were statistically decreased in CSBHT-treated mice compared with PBS-treated mice, suggesting that CSBHT inhibits Th1 responses. In accordance with the reduction in the IFN- γ level, histologic findings indicated that CSBHT decreased the peribronchial inflammation in airways. Instead of increasing Th2 by suppressing Th1, CSBHT treatment suppressed both Th1 and apparent Th2 responses but not total IgE production. Recent studies have demonstrated that IFN- γ augments leukotriene D₄-mediated responses in human airway smooth muscle [32]. IFN- γ acted as a local pro-inflammatory cytokine and exerted counter-inflammatory effects on T cells under specific conditions. We suggest that CSBHT may play an important role in both airway inflammation and immune reaction independent of the Th2 response. However, the mechanism of CSBHT remains unclear.

In conclusion, CSBHT inhibited airway inflammation and remodeling in a murine model of chronic asthma. In addition, CSBHT suppressed specifically both Th1 and Th2 responses. These findings suggest

that CSBHT may effectively delay the progression of airway inflammation and remodeling. Consequently, CSBHT may be a useful CAM for the treatment of asthma. Additional studies are needed to characterize the precise mechanism of action of CSBHT in the treatment of asthma.

Acknowledgments

This work was supported by a grant from the Oriental Medicine R&D Project, Ministry of Health and Welfare, Republic of Korea (00-PJ9-PG1-CO02-0003).

References

- [1] Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000;161:1720–45.
- [2] Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;1:520–4.
- [3] Aikawa T, Shimura S, Sasaki H, Ebina M, Takishima T. Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. *Chest* 1992;101:916–21.
- [4] Jeffery PK. Morphology of the airway wall in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991;143:1152–8.
- [5] Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991;87:893–910.
- [6] Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson SA. Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma. A quantitative light and electron microscopic study. *Am Rev Respir Dis* 1992;145:890–9.
- [7] Barnes PJ. Efficacy of inhaled corticosteroids in asthma. *J Allergy Clin Immunol* 1998;102:531–8.
- [8] Ernst E. Complementary/alternative medicine for asthma: we do not know what we need to know. *Chest* 1999;115:1–3.
- [9] Jung SK, Hwang WS, Ju CY, Lee JS, Cho IH, Jung HJ. The clinical effects of Chungsangboha-tang in asthmatic patients. *J Orient Med* 2002;23:151–60.
- [10] Institute of Laboratory Animal Resources Commission of Life Sciences NRC HJ. Guide for the care and use of laboratory animals. Washington, DC: National Academy Press; 1996.
- [11] Xiu SY. The experimental method of pharmacology. Beijing: People's Public Health Publisher; 1986.
- [12] Kwak YG, Song CH, Yi HK, Hwang PH, Kim JS, Lee KS, et al. Involvement of PTEN in airway hyperresponsiveness and inflammation in bronchial asthma. *J Clin Invest* 2003;111:1083–92.
- [13] Li XM, Huang CK, Zhang TF, Teper AA, Srivastava K, Schofield BH, et al. The Chinese herbal medicine formula

- MSSM-002 suppresses allergic airway hyperreactivity and modulates TH1/TH2 responses in a murine model of allergic asthma. *J Allergy Clin Immunol* 2000;106:660–8.
- [14] Kao ST, Wang SD, Wang JY, Yu CK, Lei HY. The effect of Chinese herbal medicine, xiao-qing-long tang (XQLT), on allergen-induced bronchial inflammation in mite-sensitized mice. *Allergy* 2000;55:1127–33.
- [15] Temelkovski J, Hogan SP, Shepherd DP, Foster PS, Kumar RK. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax* 1998;53:849–56.
- [16] Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N Engl J Med* 1991;325:1067–71.
- [17] Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989;320:271–7.
- [18] Schleimer RP, Bochner BS. The effects of glucocorticoids on human eosinophils. *J Allergy Clin Immunol* 1994;94:1202–13.
- [19] Gonzalo JA, Lloyd CM, Kremer L, Finger E, Martinez-A C, Siegelman MH, et al. Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. *J Clin Invest* 1996;98:2332–45.
- [20] Blyth DI, Wharton TF, Pedrick MS, Savage TJ, Sanjar S. 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;23:241–6.
- [21] 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;279:1120–8.
- [22] Jeffery P, Zhu J. Mucin-producing elements and inflammatory cells. *Novartis Found Symp* 2002;248:51–68.
- [23] Rogers DF. Airway goblet cell hyperplasia in asthma: hypersecretory and anti-inflammatory? *Clin Exp Allergy* 2002;32:1124–7.
- [24] Lundgren JD, Vestbo J. The pathophysiological role of mucus production in inflammatory airway diseases. *Respir Med* 1995;89:315–6.
- [25] Chu HW, Halliday JL, Martin RJ, Leung DY, Szefer SJ, Wenzel SE. Collagen deposition in large airways may not differentiate severe asthma from milder forms of the disease. *Am J Respir Crit Care Med* 1998;158:1936–44.
- [26] Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000;105:399–408.
- [27] Heinzel FP, Sadick MD, Holaday BJ, Coffman RL, Locksley RM. Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *J Exp Med* 1989;169:59–72.
- [28] Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, et al. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991;87:1541–6.
- [29] Corrigan CJ, Haczku A, Gemou-Engesaeth V, Doi S, Kikuchi Y, Takatsu K, et al. CD4 T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5. Effect of glucocorticoid therapy. *Am Rev Respir Dis* 1993;147:540–7.
- [30] Hamelmann E, Cieslewicz G, Schwarze J, Ishizuka T, Joetham C, Heusser C, et al. Anti-interleukin 5 but not anti-IgE prevents airway inflammation and airway hyperresponsiveness. *Am J Respir Crit Care Med* 1999;160:934–41.
- [31] Flaishon L, Topilski I, Shoseyov D, Hershkovich R, Fireman E, Levo Y, et al. Cutting edge: anti-inflammatory properties of low levels of IFN-gamma. *J Immunol* 2002;168:3707–11.
- [32] Amrani Y, Moore PE, Hoffman R, Shore SA, Panettieri Jr RA. Interferon-gamma modulates cysteinyl leukotriene receptor-1 expression and function in human airway myocytes. *Am J Respir Crit Care Med* 2001;164:2098–101.