

Original article

Comparison of effect of granules and herbs of Bu-Shen-Yi-Qi-Tang on airway inflammation in asthmatic mice

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Keywords: *Bu-Shen-Yi-Qi-Tang; traditional Chinese medicine; asthma; airway inflammation*

Background Bu-Shen-Yi-Qi-Tang (BSYQT), which is prescribed on the basis of clinical experience, is commonly used in clinics of traditional Chinese medicine (TCM) for asthma treatment. The components of BSYQT include *Radix Astragali* (RA), *Herba Epimedii* (HE) and *Radix Rehmanniae* (RR). The aim of this study was to compare the effect of granules and herbs of BSYQT on airway inflammation in asthmatic mice.

Methods Sixty female BALB/c mice were randomly divided into the normal control (NC) group, asthmatic group (A), decoction of granules of BSYQT treatment group (GD), decoction of herbs of BSYQT treatment group (HD), and dexamethasone treatment group (DEX). The mouse asthmatic model was induced by ovalbumin (OVA) sensitization and challenge. GD and HD of BSYQT as well as DEX were prepared and administered by intragastric infusion. Airway hyperresponsiveness (AHR) to methacholine (Mch), lung histopathology analysis, inflammatory mediators in serum (IL-4, IL-5, IL-17A, IFN- γ , and eotaxin) and in lung (IL-4, IL-5, IFN- γ , and eotaxin) were selected for investigation and comparison.

Results Both GD and HD treatment could decrease airway resistance (R_L) and increase dynamic compliance (C_{dyn}) to Mch compared with the A group ($P < 0.05$). HD treatment was more effective in R_L reduction than Mch at doses of 3.125 and 6.25 mg/ml ($P < 0.05$) and in C_{dyn} increase at Mch doses of 6.25 and 12.5 mg/ml ($P < 0.05$). There were no marked differences in R_L reduction and C_{dyn} improvement between mice in HD and DEX groups ($P > 0.05$). Both GD and HD treatment markedly attenuated lung inflammation ($P < 0.05$), and HD treatment demonstrated more significant therapeutic function in alleviating lung inflammation than that of GD and DEX treatment ($P < 0.05$). Both GD and HD treatment resulted in a significant reduction in IL-4 and IL-17A levels and an increase in the IFN- γ level in serum compared with the A group ($P < 0.05$). The effect of HD in lowering the IL-4 and IL-17A level was significantly greater than that of GD ($P < 0.05$), and was not significantly different from DEX ($P > 0.05$). HD treatment significantly reduced the serum level of IL-5 and eotaxin compared with the A group ($P < 0.05$), however, mice in the GD treatment group did not demonstrate this effect. GD and HD treatment significantly reduced IL-4 and eotaxin mRNA expression compared with the A group ($P < 0.05$). HD treatment significantly reduced IL-5 mRNA expression compared with the A group ($P < 0.05$). There was a significant difference between the GD and HD treatment groups in reducing IL-5 and eotaxin mRNA expression ($P < 0.05$). HD treatment was more effective in down-regulation of IL-5 in serum and eotaxin level both in serum and lung than DEX ($P < 0.05$). Compared with the A group, an obvious increase in mRNA expression of IFN- γ was observed in both the GD and HD treatment groups ($P < 0.05$). However, the effect of HD treatment on increase of IFN- γ mRNA expression was more apparent than GD and DEX treatment ($P < 0.05$).

Conclusions Both GD and HD treatment could decrease AHR, attenuate lung inflammation, reduce IL-4, IL-5, IL-17A, and eotaxin levels and increase IFN- γ levels in asthmatic mice. HD treatment manifests more remarkable inhibitory effects on asthmatic inflammation than GD treatment, which could provide a guide for further research on the screening of the material basis of the best anti-inflammatory effect of BSYQT.

Chin Med J 2014;127 (22): 3957-3962

Asthma is a common chronic airway inflammatory disorder which already affects an estimated 300 million individuals¹ and the prevalence of asthma is increasing.^{2,3} The major characteristics of asthma are airway inflammation, airway hyperresponsiveness (AHR), and airway remodeling; with airway inflammation considered to be the basic pathological change and one of the key pathological mechanisms of repeated attacks of asthma. Corticosteroids and β_2 -adrenoceptor agonist treatments are significantly effective in relieving asthma symptoms.^{4,5} However, there are still a number of people with asthma symptoms which are difficult to control and continue to

DOI: 10.3760/cma.j.issn.0366-6999.20140758

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This work was supported by grants from the National Basic Science Program of China (No.2009CB523000), the National Natural Science Program of China (No.81173390/H2902) and the Shanghai Inheritance Base of Yun's Converged Traditional and Western Medicine.

experience persistent symptoms and asthma exacerbations.⁶ Bu-Shen-Yi-Qi-Tang (BSYQT), which is prescribed on the basis of clinical experience, is commonly used in clinics of traditional Chinese medicine (TCM) for asthma treatment. The components of BSYQT include *Radix Astragali* (RA), *Herba Epimedii* (HE), and *Radix Rehmanniae* (RR). Our previous studies revealed that BSYQT could increase the asthma control score, decrease daily asthma symptoms, and improve lung function. However, the variable effects of BSYQT from a granules decoction (GD) and herbs decoction (HD) on the airway inflammation of asthmatic mice have not been studied and compared. This study aimed to compare the effects of GD and HD of BSYQT on airway inflammation in asthmatic mice.

METHODS

Mice

Pathogen-free, six-week-old female BALB/c mice (14–6 g) were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. and experiments were performed following the guidelines approved by the Committee on the Ethics of Animal Experiments of Fudan University.

Reagents

Ovalbumin (OVA, grade II and V), aluminum hydroxide ($\text{Al}(\text{OH})_3$) and methacholine (Mch) were purchased from Sigma-Aldrich. Herbs of RA, HE and RR were purchased from Shanghai Kangqiao Traditional Chinese Medicine Co. Ltd. Granules of RA, HE and RR were purchased from Jiangyin Tianjiang Pharmaceutical Co. Ltd. Dexamethasone (DEX) was purchased from Chenxin Pharmaceutical Co. Ltd. Mouse eotaxin ELISA kit (EK-033-40) was purchased from Phoenix, IL-17A (432507) and IL-4 (431107) were from BioLegend, and IL-5 (MBS355244) and IFN- γ (MBS262163) were purchased from Mybiosource. TriZol Reagent was purchased from Invitrogen. SYBR Green kit was purchased from TaKaRa. RevertAidTM First Strand cDNA Synthesis kit was purchased from Fermentas.

Asthmatic model establishment and treatment

Sixty female BALB/c mice were randomly divided into 5 groups (12 mice/group). Except for the normal control (NC) group, mice were sensitized by multiple intraperitoneal (i.p.) injections of OVA at a dose of 20 μg OVA adsorbed to 2 mg $\text{Al}(\text{OH})_3/0.5$ ml saline solution on days 0, 7, 14 and 21. Starting on day 25, mice were challenged by inhalation of 3% (w/v) OVA nebulized solution, with an ultrasonic nebulizer, to challenge their airways (402AI, Yuyue medical equipment Co. Ltd., Jiangsu, China) for 30 minutes three times/week for 8 consecutive weeks.⁷ Mice in the NC group were sensitized with saline solution and exposed to a saline aerosol.

From day 24, mice in the NC and asthma model (A) groups were intragastrically administrated 0.3 ml of saline solution, mice of the other three groups were intragastrically administrated 0.3 ml of GD, HD (12 g/kg body weight) and DEX (1 mg/kg body weight) one hour prior to the OVA

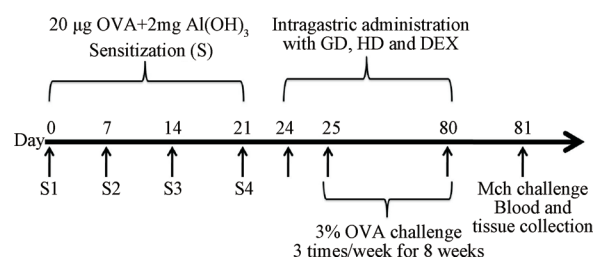


Figure 1. Experimental protocol for mouse asthma model establishment and treatment.

challenge per day for 8 consecutive weeks (Figure 1).

Measurement of AHR

AHR was evaluated by measuring the changes of airway resistance (R_L) and lung dynamic compliance (C_{dyn}) by whole-body and invasive plethysmography (Buxco Electronics Inc., NY, USA). Briefly, mice were anesthetized with pentobarbital sodium (50 mg/kg) by i.p. injection 24 hours after the last OVA challenge. A 2 mm incision was made in the trachea after the tracheostomy and the tracheal tube was inserted with a suture around the trachea to prevent tracheal tube removal and air leak. Mice were put into the body plethysmograph chamber and the inserted tracheal tube was connected to the ventilator. PBS and progressive doses of Mch (3.125, 6.25 and 12.5 mg/ml) were challenged from the ventilator through an ultrasonic nebulizer after a stable baseline airway pressure was reached.

Blood collection

Immediately after the AHR measurement, blood collection was conducted from the orbital venous plexus; blood was stored at 4°C for 2 hours and then centrifuged at $5000 \times g$ at 4°C for 15 minutes. The serum was collected, repackaged and stored at -80°C for ELISA assays.

Pulmonary histological analysis

The right lobe of the lung was removed, fixed in 4% paraformaldehyde for 24 hours and embedded in paraffin for histopathology analysis. Lung tissues were cut into 2 μm thick sections, stained with hematoxylin-eosin (HE), and examined for airway inflammation changes by light microscopy.

Inflammatory mediators assay in serum

The amounts of IL-4, IL-5, IL-17A, INF- γ , and eotaxin in serum were measured by ELISA-kit according to the manufacturer's instructions.

Quantitative real-time RT-PCR (qPCR) analysis

For analysis of IL-4, IL-5, INF- γ , and eotaxin mRNA expression, total RNA extraction of the left lung was performed with TriZol Reagent following the manufacturer's protocol. cDNA was synthesized from the total RNA using the RevertAidTM First Strand cDNA Synthesis kit following the instructions provided by the manufacturer. The mRNA expression was determined by using qPCR through SYBR Green kit. Primers used are

Table 1. Primer sequences used for qPCR amplification

Gene	Sequence
IL-4 (NM_021283.2)	Forward primer: 5'-CAGCAACGAAGAACCACCA-3' Reverse primer: 5'-AATCCAGGCATCGAAAAGCC-3'
IL-5 (NM_010558.1)	Forward primer: 5'-CAGTGTGAATGAGAGCCAGC-3' Reverse primer: 5'-TTCTGTTGGCATGGGAGT-3'
IFN- γ (NM_008337.3)	Forward primer: 5'-AGCTCTTCCTCATGGCTGT-3' Reverse primer: 5'-TCCTTTGCCAGTTCCTCCA-3'
Eotaxin (NM_011330.3)	Forward primer: 5'-CCCAGAGCCTAAGAACTGCT-3' Reverse primer: 5'-GCGACTGGTGCTGATATTCC-3'
GAPDH (NM_008084.2)	Forward primer: 5'-GTGGAGTCTACTGGTGTCTTC-3' Reverse primer: 5'-CTCAGTGTCCACCACCCT-3'

presented in Table 1. The fold change in expression of each gene was calculated using the $\Delta\Delta C_t$ method, with the housekeeping gene GAPDH mRNA as an internal control.

Statistical analysis

Data are expressed as the mean $\pm$ standard deviation (SD). Statistical analyses were performed by one-way analysis of variance (ANOVA) for multiple comparisons followed by the LSD test for comparisons between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of GD and HD of BSYQT on AHR to Mch in asthmatic mice

The effect of GD and HD of BSYQT on AHR to Mch was evaluated by measuring the changes of R_L and Cdyn. As shown in Figure 2A, our results demonstrated that OVA challenge significantly increased R_L to increasing doses of Mch compared with the NC group ($P < 0.05$). Both GD and HD treatment decreased R_L to Mch compared with the A group. However, HD treatment significantly decreased R_L at the three Mch doses ($P < 0.05$), and GD treatment only significantly decreased R_L at Mch doses of 6.25 and 12.5 mg/ml ($P < 0.05$). Although both GD and HD treatment decreased R_L at Mch doses of 3.125 and 6.25 mg/ml compared with the A group, there was a significant difference in their efficiency in R_L reduction ($P < 0.05$). As the positive control drug, DEX treatment significantly decreased R_L to increasing doses of Mch compared with asthmatic mice ($P < 0.05$). There was not a marked difference in R_L reduction between mice treated with HD

and DEX ($P > 0.05$).

As shown in Figure 2B, OVA challenge significantly decreased Cdyn to increasing doses of Mch compared with the NC group ($P < 0.05$). Both GD and HD treatment could increase Cdyn to Mch compared with the A group ($P < 0.05$). Although both GD and HD treatment increased Cdyn at Mch doses of 6.25 and 12.5 mg/ml compared with the A group, the efficiency in Cdyn promotion of HD was markedly more significant than that of GD ($P < 0.05$). Mice in the DEX group manifested a greater increase of Cdyn than that of the asthmatic mice ($P < 0.05$). The effect of HD on Cdyn improvement was the same as that of DEX ($P < 0.05$).

Effects of GD and HD of BSYQT on pulmonary pathology of asthmatic mice

As shown in Figure 3, OVA sensitization and challenge resulted in large number of inflammatory cells infiltrating, secreted mucus, and epithelial damage in the lung compared with mice in the NC group. GD, HD, and DEX treatment markedly attenuated lung inflammation ($P < 0.05$). It is worth noting that HD treatment demonstrated better alleviation of inflammation than that of GD and DEX treatment ($P < 0.05$).

Effects of GD and HD of BSYQT on inflammatory cytokine levels in serum of asthmatic mice

We also examined the level of IL-4, IL-5, IL-17A, INF- γ , and eotaxin in serum of mice in each group. There was a significant increase in the serum level of IL-4, IL-5, IL-17A, and eotaxin compared with the NC group ($P < 0.05$). A significant reduction in IL-4 and IL-17A levels in serum was observed in the GD, HD and DEX treatment groups compared with the A group ($P < 0.05$). The effect of HD in lowering IL-4 and IL-17A levels was more prominent than that of GD ($P < 0.05$). HD treatment significantly reduced serum levels of IL-5 and eotaxin compared with the A group, however, mice in the GD treatment group did not demonstrate this effect. DEX treatment remarkably reduced IL-5 levels compared with the asthmatic mice ($P < 0.05$). HD treatment was more effective in lowering IL-5 expression than was DEX ($P < 0.05$). OVA challenge

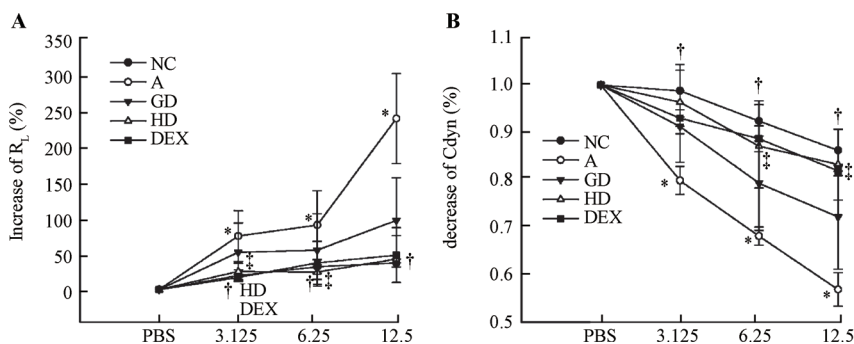


Figure 2. Effects of GD and HD of BSYQT on AHR to Mch. **A:** Effects of GD and HD of BSYQT on R_L . **B:** Effects of GD and HD of BSYQT on Cdyn. R_L and Cdyn are expressed as percentage change from the baseline value. Data is shown as mean $\pm$ SD. * $P < 0.05$, compared with NC group. † $P < 0.05$, compared with A group. ‡ $P < 0.05$, HD group vs GD group.

resulted in a remarkable decrease in IFN- γ in serum compared with mice in the NC group ($P < 0.05$). GD, HD and DEX treatment markedly elevated IFN- γ levels compared with mice in the A group ($P < 0.05$), however, there was no significant difference in the IFN- γ level among the GD, HD, and DEX treatment groups ($P < 0.05$).

Effects of GD and HD of BSYQT on mRNA expression of inflammatory cytokines in lung tissue of asthmatic mice

The effects of GD and HD of BSYQT on expression of IL-4, IL-5, INF- γ , and eotaxin mRNA were further compared. As shown in Figure 5, OVA sensitization and challenge

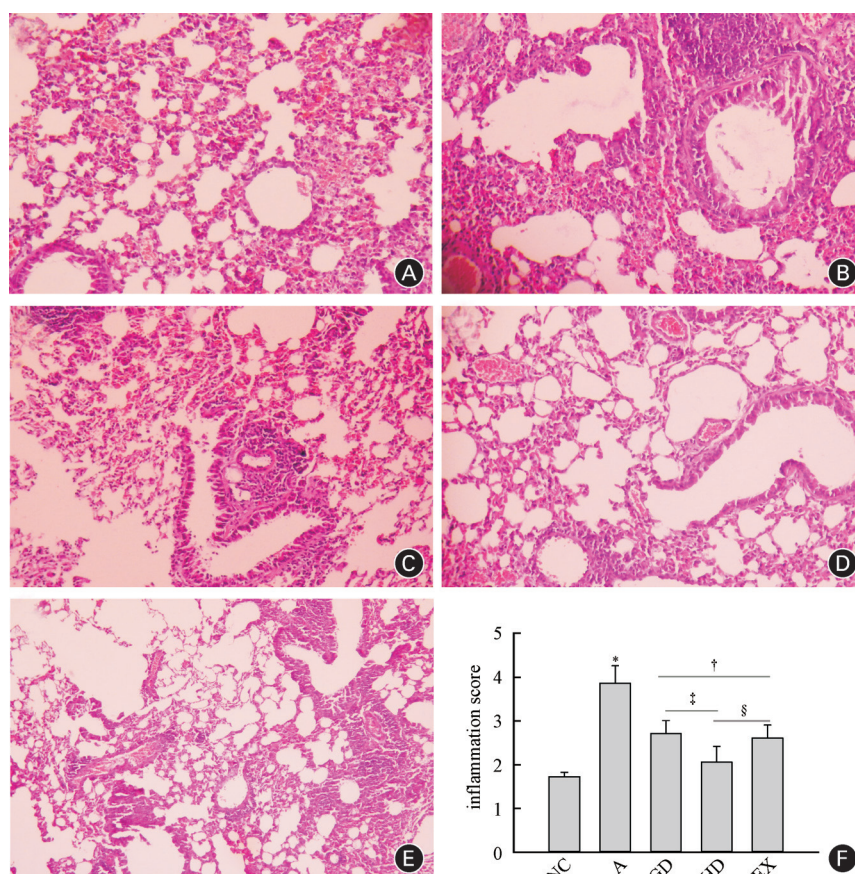


Figure 3. A-E: Lung inflammation changes of mice in each group. The lung tissue sections were stained with H&E and examined at 100× by light microscopy. Six mice were in each group and six randomly selected fields in each mouse were analyzed. **F:** Inflammatory scores in each group. The inflammatory scores in each group were assessed and data are shown as means±SD. * $P < 0.05$, compared with NC group. † $P < 0.05$, compared with A group. ‡ $P < 0.05$, HD group vs GD group. § $P < 0.05$, compared with DEX group.

increased IL-4, IL-5, and eotaxin mRNA expression levels and decreased IFN- γ mRNA expression level significantly in the lungs compared with the NC group ($P < 0.05$). GD and HD treatment significantly reduced IL-4 and eotaxin mRNA expression compared with the A group ($P < 0.05$). HD and DEX treatment significantly reduced IL-5 mRNA expression compared with the A group ($P < 0.05$). There was a significant difference between the GD and HD treatment groups in reducing IL-5 and eotaxin mRNA expression levels ($P < 0.05$). Compared with the A group, an obvious increase in mRNA expression of IFN- γ was observed in the GD, HD, and DEX treatment groups ($P < 0.05$). However, the effect of HD treatment on increase of IFN- γ mRNA expression was greater than that of GD and DEX treatment ($P < 0.05$).

DISCUSSION

In this study, the effect of GD and HD of BSYQT on airway inflammation in asthmatic mice was investigated and compared. AHR to Mch, lung histopathology analysis, inflammatory mediators in serum (IL-4, IL-5, IL-17A, IFN- γ , and eotaxin) and lung (IL-4, IL-5, IFN- γ , and

eotaxin) were selected for investigation and comparison.

Excessive airway constriction following provocative stimuli is a prominent feature of clinical asthma.⁸ In our study, OVA challenge resulted in markedly increased R_L and decreased Cdyn, and both GD and HD treatment reduced R_L and increased Cdyn, indicating the relief of AHR by GD and HD treatment. At doses of 3.125 and 6.25 mg/ml, the effect of HD on R_L was more obvious than that of GD, and at doses of 6.25 and 12.5 mg/ml, the effect of HD on Cdyn was greater than that of GD, indicating a greater therapeutic effect of HD than GD on AHR. DEX is the most frequently used agent in the clinic for asthma treatment. There were no marked differences in R_L reduction and Cdyn improvement between mice in the HD and DEX groups, indicating the obvious reduction effect of AHR in asthma. There are always inflammatory cells infiltrating into the airways and the lungs during chronic asthma.⁹ We compared the effect of GD and HD on changes in pulmonary pathology and our results proved that both GD and HD treatment attenuated lung inflammation. There was a remarkable difference in attenuating lung inflammation between the GD and HD treatment groups, and mice in the HD treatment group manifested less inflammatory damage, which was more obvious than with DEX treatment.

Asthma is traditionally viewed as an eosinophilic airway inflammatory disorder. The focus on eosinophilia in part reflects a view of asthma as being associated with an immune response biased towards a Th2 response, which is characterized by the production and secretion of Th2 cytokines such as interleukin IL-4, IL-5, and IL-13.^{6,10} Dysregulation of Th1/Th2 cytokines production is considered to be one of the mechanisms of asthma.¹¹ In this study, IL-4 and IL-5 were selected for comparison of the effect of GD and HD of BSYQT on airway inflammation in asthma. Our results demonstrate that both GD and HD treatment are effective in reducing IL-4 and IL-5 levels, and HD treatment is more effective than GD treatment. IL-4 is involved in differentiation and stimulation of Th2 cells, synthesis of IgE, and activation of macrophages.¹² IL-5 is reported to induce genes involved in the growth, survival, and activation of eosinophils¹³⁻¹⁶ and sputum IL-5 protein levels are reported to correlate with frequent asthma exacerbations.¹⁷ The reduction in IL-4 and IL-5 during GD and HD treatment might account for its effect in attenuating inflammation in asthma. The mass of evidence supports a role for Th1 cells in asthma and IFN- γ is the signature cytokine of Th1 cells. In a chronic OVA exposure model,

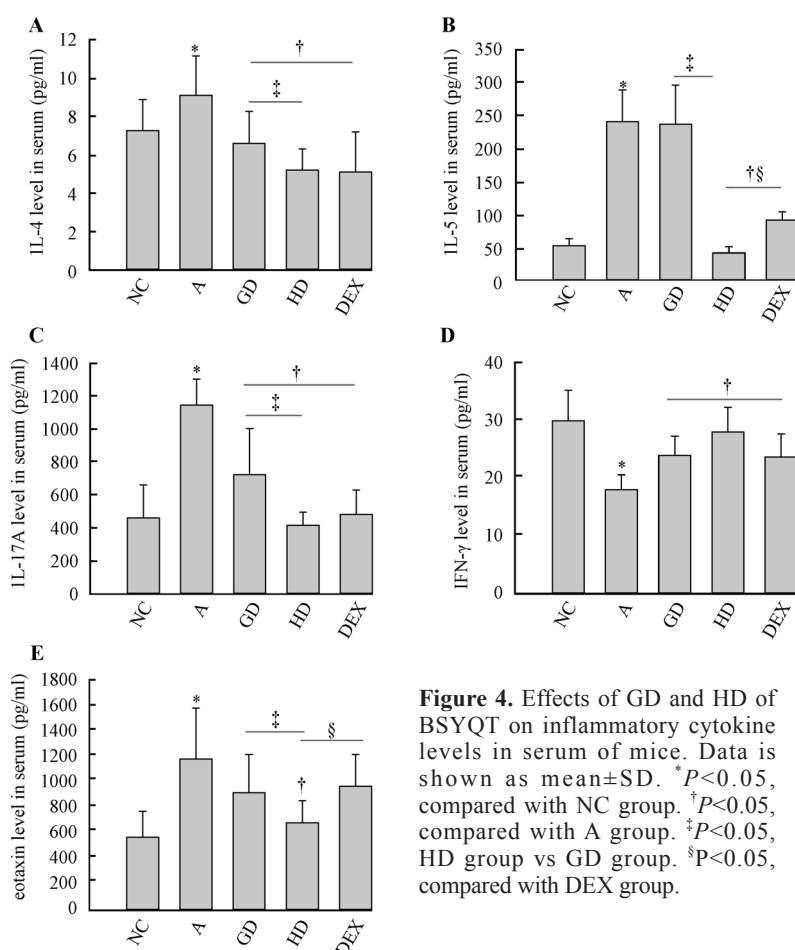


Figure 4. Effects of GD and HD of BSYQT on inflammatory cytokine levels in serum of mice. Data is shown as mean±SD. * $P < 0.05$, compared with NC group. † $P < 0.05$, compared with A group. ‡ $P < 0.05$, HD group vs GD group. § $P < 0.05$, compared with DEX group.

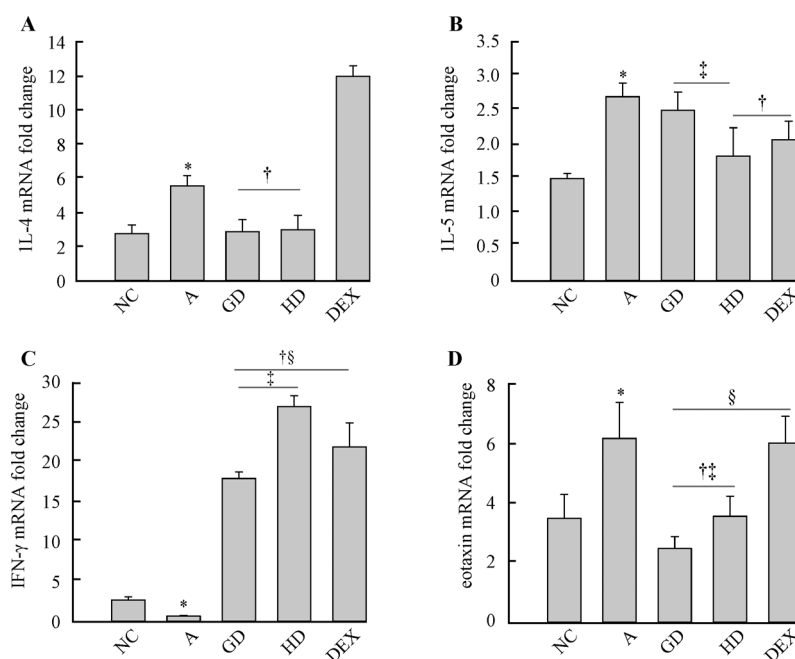


Figure 5. Effects of GD and HD of BSYQT on mRNA expression levels in the lung tissue of mice. The final data was normalized to GAPDH and presented as fold induced change compared with the expression level in NC mice. Data is shown as means ± SD values. * $P < 0.05$, compared with NC group. † $P < 0.05$, compared with A group. ‡ $P < 0.05$, HD group vs GD group. § $P < 0.05$, compared with DEX group.

mice deficient in IFN- γ and IFN- γ receptor 1 (IFN- γ R1) failed to develop increased airway resistance.¹⁸ Our results reveal that the Th1 cytokine IFN- γ is decreased during asthma and both GD and HD treatment could elevate IFN- γ mRNA expression and serum level, and HD treatment has a better effect in increasing IFN- γ mRNA expression than GD and DEX. Increased expression of IL-17 has been found in asthma¹⁹ and was most closely associated with asthma severity.^{20,21} An increase in IL-17A in serum was observed in both GD and HD treatment groups, and the increase in the HD treatment group was more remarkable than that in the GD group, indicating a stronger role of HD in lowering IL-17A. The levels of the chemokine eotaxin are markedly increased in sputum and plasma of asthmatic patients and correlated with the percentage of eosinophils in induced sputum.²² It is noted that HD treatment was more effective in down-regulation of IL-5 and eotaxin expression as well as up-regulation of IFN- γ mRNA levels than was DEX. The variety of chemical components in HD may account for its superiority to DEX. Our study proved that both GD and HD treatment could reduce eotaxin mRNA expression and serum level. However, inconsistent results were found with the effects of GD and HD on eotaxin between mRNA and serum levels. At the level of mRNA expression, the effect of GD treatment was more obvious than that of HD, on the contrary the effect of HD treatment was more marked than that of GD at the serum level. This indicates there is some posttranslational modification process in the effect of GD and HD on eotaxin expression.

In summary, the present study indicates that both GD and HD treatment could reduce airway inflammation in asthmatic mice, and HD treatment has greater inhibitory effects on inflammation, which may due to differences in the active ingredients between GD and HD. Further work will be done to ascertain the differences in the active ingredients between GD and HD.

REFERENCES

1. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;31:143-178.
2. Duramad P, Holland NT. Biomarkers of immunotoxicity for environmental and public health research. *Int J Environ Res Public Health* 2011;8:1388-1401.
3. Spergel JM. Epidemiology of atopic dermatitis and atopic march in children. *Immunol Allergy Clin*

- North Am 2010;30:269-280.
4. Holgate ST. Immune circuits in asthma. *Curr Opin Pharmacol* 2013;13:345-350.
 5. Nagai H. Recent research and developmental strategy of anti-asthma drugs. *Pharmacol Ther* 2012;133:70-78.
 6. Poon AH, Eidelman DH, Martin JG, Laprise C, Hamid Q. Pathogenesis of severe asthma. *Clin Exp Allergy* 2012;42:625-637.
 7. Nials AT, Uddin S. Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis Mod Mech* 2008;1:213-220.
 8. Reddy AT, Lakshmi SP, Reddy RC. Murine model of allergen induced asthma. *J Vis Exp: JoVE* 2012:e3771.
 9. Jin R, Guo S, Wang MY, Li YH, Wu LX, Ma H, et al. Administration of mycobacterial Ag85A and IL-17A fusion protein attenuates airway inflammation in a murine model of asthma. *Int Immunopharmacol* 2013;17:1067-1074.
 10. Nair P. What is an "eosinophilic phenotype" of asthma? *J Allergy Clin Immunol* 2013;132:81-83.
 11. Fulkerson PC, Rothenberg ME. Targeting eosinophils in allergy, inflammation and beyond. *Nat Rev Drug Discov* 2013;12:117-129.
 12. Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and Il4 locus accessibility. *Annu Rev Immunol* 2006;24:607-656.
 13. Dyer KD, Percopo CM, Rosenberg HF. IL-33 promotes eosinophilia in vivo and antagonizes IL-5-dependent eosinophil hematopoiesis ex vivo. *Immunol Lett* 2013;150:41-47.
 14. Wilson TM, Maric I, Shukla J, Brown M, Santos C, Simakova O, et al. IL-5 receptor alpha levels in patients with marked eosinophilia or mastocytosis. *J Allergy Clin Immunol* 2011;128:1086-1092.
 15. Takatsu K, Nakajima H. IL-5 and eosinophilia. *Curr Opin Immunol* 2008;20:288-294.
 16. Leitch VD, Strudwick XL, Matthaei KI, Dent LA, Cowin AJ. IL-5-overexpressing mice exhibit eosinophilia and altered wound healing through mechanisms involving prolonged inflammation. *Immunol Cell Biol* 2009;87:131-140.
 17. Xu J, Jiang F, Nayeri F, Zetterstrom O. Apoptotic eosinophils in sputum from asthmatic patients correlate negatively with levels of IL-5 and eotaxin. *Respir Med* 2007;101:1447-1454.
 18. Yu M, Morgan AA, Mukai K, Butte AJ, Tsai M, Galli SJ. Identification of an IFN- γ /mast cell axis in a mouse model of chronic asthma. *J Clin Invest* 2011;121:3133-3143.
 19. Zhao Y, Yang J, Gao YD, Guo W. Th17 immunity in patients with allergic asthma. *Int Arch Allergy Immunol* 2010;151:297-307.
 20. Al-Ramli W, Prefontaine D, Chouiali F, Martin JG, Olivenstein R, Lemiere C, et al. Hamid Q: T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J Allergy Clin Immunol* 2009;123:1185-1187.
 21. Agache I, Ciobanu C, Agache C, Anghel M. Increased serum IL-17 is an independent risk factor for severe asthma. *Respir Med* 2010;104:1131-1137.
 22. Daldegan MB, Teixeira MM, Talvani A. Concentration of CCL11, CXCL8 and TNF- α in sputum and plasma of patients undergoing asthma or chronic obstructive pulmonary disease exacerbation. *Braz J Med Biol Res* 2005;38:1359-1365.

(Received March 31, 2014)

Edited by Wang De