

Occupational asthma and rhinitis caused by multiple herbal agents in a pharmacist

Soo-Keol Lee*, MD; Hyeon-Kyeong Cho, MD*; Sang-Heon Cho, MD, PhD†; Sun-Sin Kim, MD*; Dong-Ho Nahm, MD, PhD*; and Hae-Sim Park, MD, PhD*

Background: Herb agents have been widely used for centuries in the Orient and they have been cultivated throughout Asia. There have been a few cases of occupational allergy caused by herb materials. We report a case of occupational asthma and rhinitis caused by six herb materials in a pharmacist working at a pharmacy.

Objective: We sought the role of immediate hypersensitivity in herbal agent-induced asthma in a pharmacist.

Methods and Results: The patient had strong positive responses on skin prick test to extracts of six herb materials: Chunkung (*Cnidii rhizoma*), Banha (*Pinellia ternata*), Sanyak (*Dioscorea radix*), Kangwhal (*Ostericum koreanum*), Danggui (*Angelica radix*), and Kunkang (*Zingiberis rhizoma*). Bronchoprovocation tests showed an early asthmatic response to Danggui extract. Serum specific IgE antibodies to Chunkung, Banha, and Sanyak were detected by ELISA with no specific IgE bindings to Kangwhal, Danggui, and Kunkang extracts. Twelve percent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and IgE immunoblotting revealed one IgE binding component (60 kD) within Chunkung extract, two (10, 25 kD) in Banha, and four (33, 34, 65, 98 kD) in Sanyak. Basophil histamine release test revealed that Danggui extract could release a greater amount of histamine from basophils in the patient than in a healthy control.

Conclusions: Chunkung, Banha, and Sanyak may induce IgE-mediated bronchoconstriction in an exposed worker, and Danggui can cause bronchoconstriction by direct histamine-releasing effect from mast cells in a sensitized patient.

Ann Allergy Asthma Immunol 2001;86:469–474.

INTRODUCTION

There have been a few reports of occupational asthma induced by herb materials.^{1–3} An IgE-mediated mechanism has been implicated in most cases including Brazil ginseng, Sanyak and Banha. Six herb materials (*Ostericum koreanum*, *Angelica radix*, *Cnidii rhizoma*, *Dioscorea radix*, *Pinellia ternata*, *Zingiberis rhizoma*) to which our patient was sensitized have been

widely used for centuries in the Orient and they have been cultivated throughout Far East Asia, including Korea. Three of them, Kangwhal, Danggui, and Chunkung, belong to the Umbelliferae family, and are thought to be effective as an anti-inflammatory agent (Kangwhal), vascular volume expander (Danggui), and autonomic nervous system modulator (Chunkung). In contrast, Sanyak (*Dioscorea radix*), Banha (*Pinellia ternata*), and Kunkang (*Zingiberis rhizoma*) belong to different families, the Dioscoreaceae, Araceae, and Zingiberaceae, respectively. Sanyak is thought to be effective in supporting gastrointestinal function. Banha is used as an expectorant and cough suppressant. Kunkang is used as a digestive.⁴ To the best of our knowledge, there are no published data of occupational asthma caused by Danggui with sensitization to six different herbal agents.

In this study, we report a case of occupational asthma and rhinitis induced by various mechanisms concurrently sensitized to multiple herbal agents in a pharmacist.

CASE REPORT

The patient was a 35-year-old male pharmacist. Fifteen years earlier, he had been employed at a relative's herb materials shop where his responsibilities included chopping many kinds of herb materials to prepare for sale. He had complained of profuse rhinorrhea and sneezing 10 years before admission. Cough and shortness of breath had started 7 years ago. These symptoms became more severe at night after handling herb materials during the day, but improved when he was on vacation. His asthma symptom severity was classified as moderate to severe on admission. He smoked a pack of cigarettes for 10 years, but had stopped 3 years ago. The total eosinophil count was 200/mm³, and total IgE level by radioimmunoassay (Diagnostic Products, Los Angeles, CA) was 1,331 IU/mL. Chest and paranasal sinus radiography revealed no abnormal findings. Methacholine bronchial challenge test showed a positive response at 0.31 mg/mL. The patient had shown negative responses to 80 common inhalant and food allergens; however, strong positive responses to six herb material extracts were noted on the skin prick test (Table 1). He gave a written informed consent which was regulated by Ajou University Hospital, Suwon, Korea.

MATERIALS AND METHODS

Preparation of Six Herbal Agent Extracts

The six herbal agents were obtained from the patient's pharmacy. All of

* Department of Allergy and Clinical Immunology, Ajou University School of Medicine, Suwon, Korea.

† Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea. This study was supported by a research grant (HMP-98-M-1-0001) from the Ministry of Health and Welfare, Republic of Korea.

Received for publication August 27, 2000.

Accepted for publication in revised form November 24, 2000.

Table 1. The Results of Skin Prick Test with Six Herb Materials and Amylase

Allergens	Skin-prick test Wheal/erythema (mm)
Kangwhal	30 × 17/72 × 48
Danggui	19 × 10/50 × 42
Chunkung	19 × 9/57 × 44
Kunkang	5 × 3/23 × 22
Sanyak	18 × 8/61 × 35
Banha	42 × 21/72 × 60
Amylase	3 × 3/4 × 4
Histamine	6 × 3/4 × 4
Control	0

them were cultivated in Korea. They were cut into small pieces and extracted into phosphate-buffered saline (PBS; pH 7.5) 1:5 wt/vol, at 4° C for 24 hours, followed by centrifugation at 4,000 rpm at 4° C for 30 minutes. The supernatant was dialyzed (the cut-off molecular weight was 6 kD) against 4 L of normal saline at 4° C for 48 hours and then used as crude extract.

Inhalation Challenge Test with Methacholine

Airway responsiveness to methacholine was performed according to a method described previously.⁵ In this study, methacholine challenge was performed 1 day before bronchoprovocation test (BPT) using Danggui extract.

BPT with Danggui Extracts

BPT was performed in a similar manner to other occupational asthma studies.⁶⁻⁹ One mL of normal saline was administered from a nebulizer 646 (Devillebiss, Somerset, PA) connected to a dosimeter (5 L/minute). The subject was asked to breathe the aerosol with tidal breathing, and this was followed by antigen extracts. The forced expiratory volume in 1 second (FEV₁) and FEF_{25%-75%} were measured with a spirometer (Multispiro, Irvine, CA) before and 10 minutes after each inhalation. Then, the FEV₁ and FEF_{25%-75%} were measured every 10 minutes during the first hour. Pulmonary function tests were performed every hour for 7 hours after the challenge.

ELISA for Specific IgE to Six Herbal Agents and ELISA Inhibition Test

The presence of specific IgE antibodies to six herb agents was determined by ELISA. Microtiter plates (Corning, New York, NY) were first coated with 100-μL extracts of six herbal agents dissolved in carbonate buffer, pH 9.6 (10 μg/well). After blocking, the wells were then incubated with 100 μL of either the patient's serum (1:5 vol/vol) or control sera (1:5 vol/vol) from seven patients who showed negative skin prick test responses to common inhalant allergens as well as six herbal agents. After washing, the immunoplate was incubated with 1:1,000 vol/vol biotin-labeled goat anti-human IgE antibody (Sigma, St. Louis, MO) followed by incubation with 1:1,000 vol/vol streptavidin-peroxidase (Sigma). After washing, 75 μL of TMB solution 3,3',5,5'-tetramethylbenzidine, one tablet in 10 mL of phosphate citrate buffer containing 2 μL of 30% hydrogen peroxide) was added as substrate, and 75 μL of 2.5 NH₂SO₄ was added to stop the reaction 5 minutes later. A calorimetric reaction was measured by the absorbancy at 450 nm on an ELISA reader. To investigate cross-reactivity between herb materials, ELISA inhibition tests were performed. In Chunkung-ELISA inhibition test, the patient's serum was preincubated for 12 hours at 4° C with Chunkung, Danggui, Banha, Sanyak, and house dust mite (HDM),

and added to a Chunkung-coated microplate. In the case of Banha, preincubation of patient's serum was done with Banha, Sanyak, Kangwhal, Chunkung, and HDM, and added to a Banha-coated microplate. For Sanyak-ELISA inhibition test the patient's serum was preincubated with Sanyak, Banha, Kangwhal, Danggui, and HDM, and then added to a Sanyak-coated microplate. The remaining steps for IgE-ELISA were performed as described previously.

SDS-PAGE and Immunoblotting

Chunkung (20 μg/well), Banha (15 μg/well) and Sanyak (8 μg/well) extracts were electrophoresed in a 12% polyacrylamide separation gel with 4% stacking gel. Proteins from the gels were stained with colloidal blue (Novex, San Diego, CA) or transferred onto a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA), which was incubated with the patient's serum diluted 1:5 vol/vol with PBS-Tween. Bound specific IgE was detected by biotin-labeled goat anti-human IgE antibody (Sigma) conjugated with streptavidine alkaline phosphatase (Sigma) followed by 0.66% 4-nitroblue tetrazolium and 0.33% 5-bromo-4-chloro-3-indolyl-phosphate (Sigma), which was used as a substrate.

Basophil Histamine Release Test

The techniques involved in preparing peripheral blood leukocytes for performing histamine release, for staging

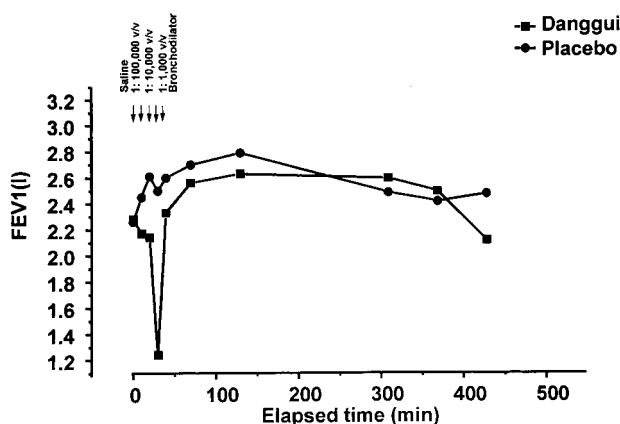


Figure 1. The results of BPTs with Danggui extract and placebo.

the histamine release reaction, and for fluorometric measurement of histamine have been described previously.^{10,11} Briefly, 20 mL of blood was obtained from the subject and mixed with 2.5 mL of 6% dextran-3% dextrose-physiologic saline and 1 mL of ethylenediaminetetraacetic acid. The mixture was allowed to sediment at room temperature for 2 hours before the leukocytes containing plasma were removed by centrifugation at 4° C and 250 *g* for 8 minutes. The leukocyte pellet was washed twice with PIPES-A (25 mmol/L piperazine-N-N'-bis-2 ethane sulfonic acid, 110 mmol/L NaCl, 5 mmol/L KCL, 40 mmol/L NaOH, and 0.03% human serum albumin adjusted to pH 7.4) and before histamine release studies, it was resuspended in PIPES-ACMD (91 mM CaCl₂, QS mM MgCl₂, 100 mg% dextrose in PIPES) of approximately 5 × 10⁶ cells/mL. One hundred μ L of the Danggui and Sanyak extract dilutions (0.001, 0.01, and 0.1 mg/mL), calcium inophore A23197 (1,3, and 7 μ m), anti-human goat IgG4 antibodies (1:100, 1:1,000, 1:10,000 vol/vol), or anti-human goat IgE antibodies (1:10, 1:100, 1:1,000 vol/vol) were added to 200 μ L of leukocyte suspension and the suspensions were incubated for 15 minutes at 37° C. Similarly, leukocyte suspensions were incubated with PIPES-ACMD alone to correct for spontaneous histamine release. Total cellular histamine concentration was measured by adding 0.1 mL of 55% trichloroacetic acid to leukocyte suspension. The tubes were centrifuged at 4° C and 250 *g* for 15 minutes and the supernatants were collected and stored at -20° C for subsequent assay of histamine. All the experiments were performed in duplicate. Histamine concentration was measured by the automated fluorometric method described earlier. Histamine release was expressed as percentage of total cellular histamine, based on the following formula:

Percentage of histamine release = $100 \times (\text{stimulated histamine release} - \text{spontaneous histamine release} / \text{total cellular histamine release} - \text{spontaneous histamine release})$

RESULTS

BPT with Danggui Extract

As shown in Figure 1, there was no significant change after inhalation of 1:100,000 and 1:10,000 dilutions of Danggui extract; however, an isolated early asthmatic reaction was noted after inhalation of 1:1,000 dilution of extract.

Detection of Specific IgE Antibodies to Six Herbal Agent Extracts and ELISA Inhibition Tests

Table 2 shows the specific IgE bindings to six herbal agents in the patient's serum and control sera. The absorbance value of control sera was derived from mean absorbance value of seven control patients showing negative results on skin prick tests with 80 common inhalant and food allergens, including six herb material extracts. In comparison to control sera, high specific IgE bindings to Chunkung, Banha, and Sanyak extracts were noted in the patient's serum. In the case of Kangwhal, Danggui, and Kunkang, little bindings were noted in both the patient's serum and control sera. Figure 2 reveals the Chunkung-, Banha-, and Sanyak-ELISA inhibition results with addition of four different herb material extracts and HDM.

Chunkung-ELISA inhibition test showed significant inhibition with addition of Chunkung, whereas no significant inhibitions were noted with addition of Danggui, Banha, Sanyak, and HDM. Banha-ELISA inhibition test showed significant inhibitions with addition of

Banha, whereas there were no significant inhibitions with addition of Sanyak, Kangwhal, Chunkung, and HDM. In Sanyak-ELISA inhibition test, there was significant inhibition with addition of Sanyak, whereas no significant inhibitions were noted with addition of Banha, Kangwhal, Danggui, and HDM.

Gel Electrophoresis and Immunoblot Analysis

To understand the protein components of Chunkung, Banha, and Sanyak extracts, the extracts were analyzed by a 12% SDS-PAGE. Figure 3A reveals the resolution of the three herb material extracts. Figure 3B shows specific IgE bindings in patient and control sera on the blotted membrane. One IgE binding component (60 kD) was detected within Chunkung extract, two (10, 25 kD) in Banha, and four (33, 34, 62, 98 kD) in Sanyak. No bindings were noted with control sera.

Basophil Histamine Release Test

Table 3 shows comparison of basophil histamine release from a healthy control and a patient with serial additions of two herbal agent extracts (Sanyak, Danggui), anti-IgE antibody, and anti-IgG4 antibody. Compared with healthy control, basophil histamine release by Sanyak extract and anti-IgE antibodies was higher in the patient, whereas there was minimal release in the control. The histamine release by anti-IgG4 antibodies was minimal in both patient and control. Danggui extract could induce histamine release from patient and control, however much greater amount of histamine release was noted in the patient.

DISCUSSION

This study demonstrated that herbal agents could induce occupational asthma in an exposed worker in a pharmacist. This result was confirmed by specific BPT.

The herbal materials have been used widely as drugs for thousands of years in the Orient. Several reports¹⁻³ have pointed out their importance as an occupational inhalant allergen among jobs, including laboratory assistants and phar-

Table 2. Specific IgE Bindings to Six Herb Material Extracts in Sera of the Patient and Controls

Allergen	Absorbance value	
	Patient	Control
Kangwhal	0.174	0.102
Danggui	0.147	0.102
Chunkung	1.173	0.109
Banha	1.105	0.060
Kunkang	0.133	0.100
Sanyak	1.183	0.108

Absorbance values of control sera were mean value of seven healthy controls.

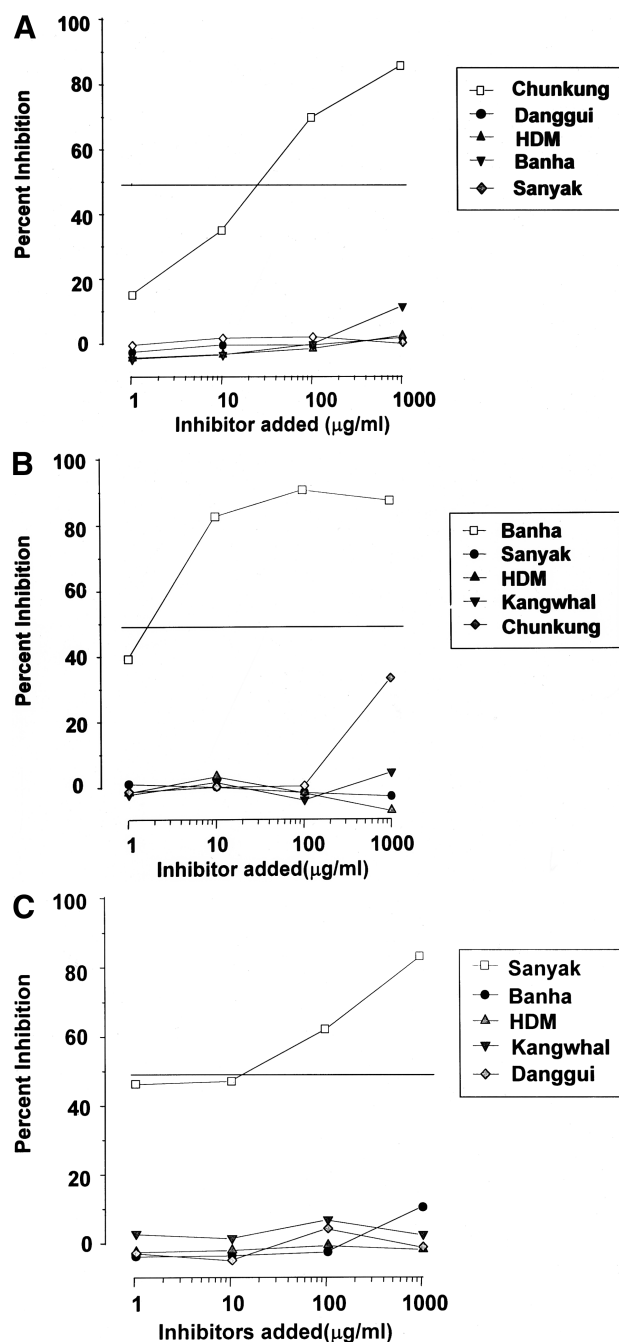


Figure 2A. Chunkung-ELISA inhibition test with addition of Chunkung, Danggui, HDM, Banha, and Sanyak extracts. 2B. Banha-ELISA. inhibition test with addition of Banha, Sanyak, HDM, Kangwhal, and Chunkung extracts. 2C. Sanyak-ELISA. inhibition test with addition of Sanyak, Banha, HDM, Kangwhal, and Danggui extracts.

maceutical employees. Our patient's job was as a pharmacist who handled herb materials. Because he had handled a lot of drug powders such as amylase, a raw material of digestives and aspirin, as

commonly prescribed antipyretics and herbal agents, we performed three consecutive BPTs with amylase and lysine-aspirin powders, as well as Danggui extract.

There were no significant changes of FEV₁ after inhalation of both amylase and lysine-aspirin solution (data not shown). The pathogenic mechanism of occupational asthma caused by Danggui may be a non-IgE mediated response, but evidence suggests a direct histamine-releasing effect from mast cells. First, skin prick test with Danggui extract showed a strong positive response while there were negligible responses in healthy controls. Second, specific IgE antibody to Danggui was undetectable by ELISA and no specific IgE binding was noted on immunoblot analysis (data not shown). Third, basophil histamine release test with Danggui extract showed complete release of histamine from basophils in the patient and some in a control. Further, the histamine release by Danggui extract was dose-dependent; the higher the concentration of Danggui, the greater the amount of histamine released.

There have been several reports about occupational allergens which have induced bronchoconstriction through direct histamine-releasing effect from mast cells. Our previous study¹¹ revealed that grain dust could induce enhanced basophil histamine-releasing effect in patients with grain dust induced occupational asthma. Although we could not attempt to further explore the mechanism of Danggui-induced histamine release, we speculated that direct basophil histamine-releasing effect by Danggui might contribute to the development of airway obstruction in exposed workers.

Regarding the mechanism of other herb materials, although BPTs with Chunkung, Banha, and Sanyak extracts were not performed, we can speculate that IgE-mediated reactions might be responsible in the case of these three herb materials, because the skin prick tests with these three herbs showed positive responses. Further, high specific IgE antibodies to each of these three herbal agents were detected by ELISA. Further, ELISA inhibition test results suggested specificity of IgE antibody bindings to each of these three herb antigens without cross-reactivities between them. The immunoblotting study using the three extracts showed specific IgE-binding com-

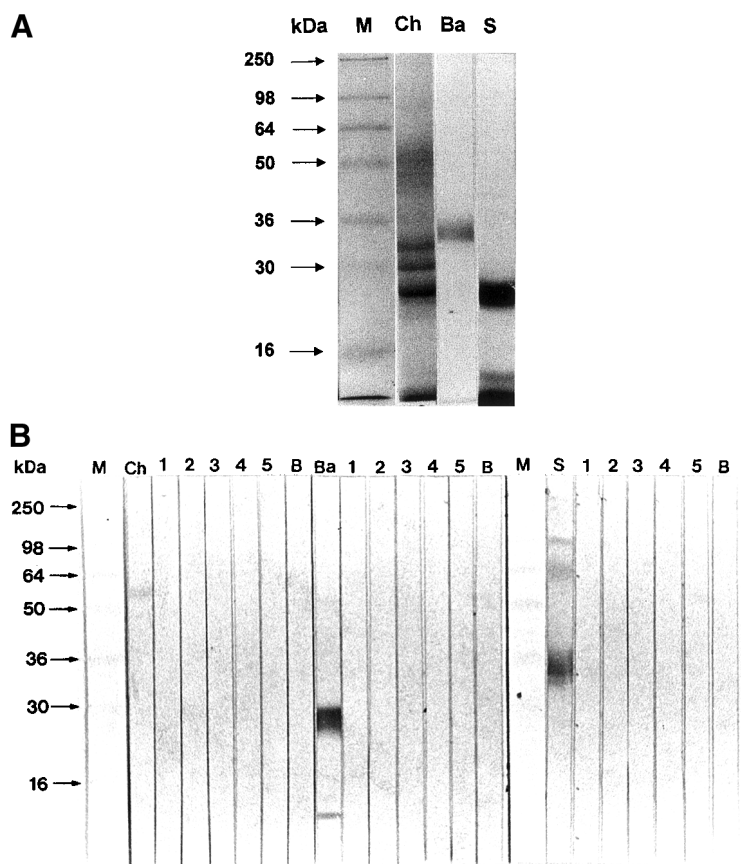


Figure 3A. 12% SDS-PAGE findings of three herbal agents. 3B. IgE-binding components of three herbal agents analyzed by immunoblotting. The standards used were myosin (250 kD), bovine serum albumin (98 kD), glutamic dehydrogenase (64 kD), alcohol dehydrogenase (50 kD), carbonic anhydrase (36 kD), myoglobin (30 kD), and lysozyme (16 kD). M: marker protein; Ch: Chunkung; Ba: Banha; S: Sanyak; B: buffer; and 1 to 5: control sera

Table 3. Results of Basophil Histamine Release by Multiple Stimulants in the Patient and Healthy Control

Stimulant		Net histamine release (%)* in	
		Healthy control	Patient
Anti-IgE (dilution, vol/vol)	1:1000	0	0.7
	1:100	34	48
	1:10	31	53
Ca ionophore (micromoles)	1	1.2	0
	3	0	0
	7	63	46
Anti-Ig G4 (dilution, vol/vol)	1:10000	0	0
	1:1000	0.6	0.4
	1:100	5.3	0
Sanyak (mg/mL)	0.001	0	59
	0.01	0	32
	0.1	0	42
Danggui (mg/mL)	0.001	0.6	16
	0.01	5.6	41
	0.1	67	100

* (Post-stimulant – spontaneous)/(after cell lysis – spontaneous) × 100.

ponents within the individual extract. These results suggest that Chunkung, Banha, and Sanyak could induce IgE-mediated bronchoconstriction in this patient. Recently, Park et al³ reported about occupational asthma caused by Banha in a pharmaceutical employee. They reported that specific IgE antibodies to Banha were undetectable by ELISA and contrary to this study, there was no IgE binding components in immunoblotting using patient's serum. This finding suggests that Banha can induce bronchoconstriction by IgE-mediated response, as well as non-IgE-mediated response.

The role of specific IgG and IgG4 antibodies in occupational asthma has been studied.¹² The exact pathogenic role of these antibodies in the development of occupational asthma has shown variable results. Some investigators suggested that these antibodies might be used as exposure markers, not having a pathogenic role in a clinical situation.^{13–15} In contrast, our previous study¹⁶ suggested a possible pathogenic role of specific IgG antibodies to human albumin conjugate in the pathogenesis of toluene diisocyanate-induced asthma. In the present study, basophil histamine release by anti-IgG4 antibody was minimal, suggesting that the possibility of specific IgG4 antibody as a sensitizing antibody was very low.

CONCLUSION

This study demonstrates that multiple herb materials can induce occupational asthma with various mechanisms, including IgE and non-IgE mediated responses, in a single exposed patient.

REFERENCES

1. Subiza J, Subiza JL, Escribano PM, et al. Occupational asthma caused by Brazil ginseng dust. *J Allergy Clin Immunol* 1991;88:731–736.
2. Park JK, Kim YK, Yoon HJ, et al. [A case of occupational asthma induced by licorice] (in Korean). *Kor J Med* 1994; 47:866–871.
3. Park HS, Kim MJ, Moon HB. Occupational asthma caused by two herb materials, *Dioscorea batatas* and *Pinellia ternata*. *Clin Exp Allergy* 1994;24: 575–581.
4. Jung BS, Shin MS. [Illustrated Large

- Dictionary of Herb Materials] (in Korean). Seoul: Younglim Co, 1990.
5. Chai H, Farr RS, Froehlich LA. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;56:323-327.
 6. Park HS, Nahm DH. Buckwheat flour hypersensitivity: an occupational asthma in noodle maker. *Clin Exp Allergy* 1996;26:423-427.
 7. Park HS, Nahm DH. New occupational allergen in a pharmaceutical industry: serratal peptidase and lysozyme chloride. *Ann Allergy Asthma Immunol* 1997;97:225-229.
 8. Suh CH, Park HS, Nahm DH, et al. Oil-seed rape allergy presented as occupational asthma in the grain industry. *Clin Exp Allergy* 1998;28:1159-1163.
 9. Kim HY, Nahm DH, Park HS, et al. Occupational asthma and IgE sensitization to cellulase in a textile industry worker. *Ann Allergy Asthma Immunol* 1999;82:223-228.
 10. Cromwell O, Durham SR, Shaw RJ, et al. Provocation test and measurements of mediators from mast cells and basophils in asthma and allergic rhinitis. In: Weir DM, ed. *Applications of Immunological Methods in Biomedical Science*. Oxford: Blackwell Scientific, 1986:127.
 11. Park H, Jung K, Kang K, et al. Enhanced basophil histamine release and neutrophil chemotactic activity predispose grain dust-induced airway obstruction. *Clin Exp Allergy* 1999;29:543-549.
 12. Tiikkainen U, Klockars M. Clinical significance of IgG subclass antibodies to wheat flour antigens in bakers. *Allergy* 1990;45:497-504.
 13. Price JA, Longbottom JL. IgG antibodies in relation to exposure to laboratory-animal allergens. *J Allergy Clin Immunol* 1989;84:520-528.
 14. Zock JP, Doekes G, Heederik D, et al. Airborne dust antigen exposure and specific IgG response in the potato processing industry. *Clin Exp Allergy* 1996;26:542-548.
 15. Park HS, Suh CH, Nahm DH, Kim HY. Presence of specific IgG antibody to grain dust does not go with respiratory symptoms. *J Korean Med Sci* 1999;14:39-44.
 16. Park HS, Kim HY, Nahm DH, et al. Specific IgG, but not specific IgE, antibodies to toluene diisocyanate-human serum albumin conjugate are associated with toluene diisocyanate bronchoprovocation test results. *J Allergy Clin Immunol* 1999;104:847-851.

Requests for reprints should be addressed to:
Dr. Hae-Sim Park
Department of Allergy and Clinical Immunology
Ajou University School of Medicine
Paldalgu Wonchondong San-5
Suwon, Korea 442-749
E-mail: hspark@madang.ajou.ac.kr