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.

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.⁵ 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
and FEF25%-75% were measured with a 470 ANNALS OF ALLERGY, ASTHMA, & IMMUNOLOGY

hours after the challenge. Tests were performed every hour for 7

ing the first hour. Pulmonary function were measured every 10 minutes dur-

method described previously.

line was performed according to a

Airway responsiveness to methacho-

were cut into small pieces and ex-

molecular weight was 6 kD) against 4

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
cients who showed negative skin

t common inhalant allergens as well as six herbal

After washing, the immuno-

incubated with Sanyak, Banha, Kangwhal, Danggui, and HDM,

to six herb agents was determined

ELISA for Specific IgE to Six Herbal

The presence of specific IgE antibo-

dissolved in carbonate buffer, pH 9.6

(10 µg/well). After blocking, the wells

of either the patient’s serum (1:5 vol/vol)

or control sera (1:5 vol/vol) from seven

agents. After washing, the immuno-

be incubated with 1:1,000 vol/vol streptavidin-peroxidase (Sigma).

material was incubated 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-phosphatase (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

<table>
<thead>
<tr>
<th>Allergens</th>
<th>Skin-prick test</th>
<th>Wheal/erythema (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kangwhal</td>
<td>30 × 17/72 × 48</td>
<td></td>
</tr>
<tr>
<td>Danggui</td>
<td>19 × 10/50 × 42</td>
<td></td>
</tr>
<tr>
<td>Chunkung</td>
<td>19 × 9/57 × 22</td>
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</tr>
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<td>5 × 3/23 × 22</td>
<td></td>
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<tr>
<td>Sanyak</td>
<td>18 × 8/61 × 35</td>
<td></td>
</tr>
<tr>
<td>Banha</td>
<td>42 × 21/72 × 60</td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>3 × 3/4 × 4</td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>6 × 3/4 × 4</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
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Table 1. The Results of Skin Prick Test with Six Herb Materials and Amylase

In this

them were cultivated in Korea. They

were cut into small pieces and ex-

tracted 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

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

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</table>
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 reports have pointed out their importance as an occupational inhalant allergen among jobs, including laboratory assistants and phar-
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 study 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 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.
These results suggest that Chunkung, Banha, and Sanyak could induce IgE-mediated bronchoconstriction in this patient. Recently, Park et al.\(^3\) 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.\(^1\) 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 study\(^16\) 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**

4. Jung BS, Shin MS. [Illustrated Large...


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