

Occupational asthma caused by two herb materials, *Dioscorea batatas* and *Pinellia ternata*

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Summary

There have been very few cases of occupational allergies caused by herb materials. In this paper, we report a case of occupational asthma and rhinitis caused by two herb materials, *Dioscorea batatas* and *Pinellia ternata*, which are known as Sanyak and Banha respectively. The patient had strong positive responses to grass pollens and cat fur, as well as to Sanyak and Banha extracts on skin-prick tests. Bronchoprovocation tests showed a dual asthmatic response to Sanyak whereas only an early asthmatic response to Banha. Serum specific IgE and IgG4 antibodies to Sanyak were detected by enzyme-linked immunosorbent assay (ELISA) but there was no specific IgE antibody binding to Banha. In order to further characterize the allergenic component of these extracts, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotting studies were also performed. Three IgE binding components (29 000, 57 000, 63 000 Da) were detected within the Sanyak extracts, but no IgE-binding component was noted within the Banha extracts. It is suggested that Sanyak can induce IgE-mediated bronchoconstriction in an exposed worker. Further studies are needed to investigate the pathogenetic mechanism triggered by Banha.

Clinical and Experimental Allergy, Vol. 24, pp. 575–581. Submitted 3 November 1993; revised 22 November 1993; accepted 15 February 1994.

Introduction

Many inhaled organic agents can cause occupational asthma in atopic and non-atopic subjects. A number of these agents are plant-derived materials, and in many cases, an IgE-mediated hypersensitivity mechanism has been implicated. The sources of inducing antigens have been found in many different parts of the plant: fruits, seeds, pollens, wood, plant exudate, leaves and roots [1–5]. Two herb materials (Sanyak and Banha) to which our patient was sensitized have been widely used for centuries in the Orient and they have been cultivated throughout Asia. Sanyak (*Dioscorea batatas*) belongs to the Dioscoreaceae family, Liliales order and its roots have been used to treat gastrointestinal problems. Banha (*Pinellia ternata*) belongs to the Araceae family, Arales order [6], and the root of Banha is used as an expectorant and cough suppressant. There have been few case reports dealing

with occupational asthma due to herb materials. Recently, Subiza *et al.* [7] reported one case of occupational asthma caused by Brazil ginseng dust, which has also been used in herb medicine.

In this paper, we report on a case of occupational rhinitis and asthma caused by herb materials, a result which was confirmed by bronchoprovocation tests. Serum specific antibodies were detected and their IgE binding components were identified.

Subjects and methods

Case summary

The patient was 25-year-old male smoker, who was admitted to the Allergy Clinic of the National Medical Center, Seoul, Korea. Three years earlier, he had been employed at a herbal medicine pharmacy and his job responsibilities included chopping many kinds of herb materials to prepare the medications. He began to cough and experience shortness of breath 1 year before this study, and suffered from profuse rhinorrhoea and sneezing in the last 2 months. He complained that the above

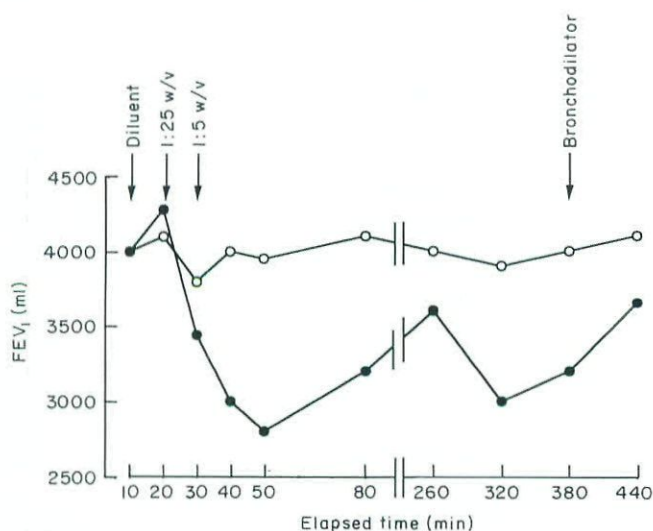
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Table 1. The result of skin-prick test and RAST

Allergens	Skin-prick test Weal/Erythema(mm)	RAST class
Cochroach	3 × 2/21 × 16	ND
Cat fur	6 × 4/25 × 24	1
<i>Dermatophagoides farinae</i>	1 × 1/17 × 17	0
<i>Dermatophagoides pteronyssinus</i>	2 × 2/18 × 15	0
Orchard grass	3 × 3/23 × 16	2
Timothy grass	7 × 7/30 × 22	0
Rye grass	4 × 3/25 × 20	ND
Ragweed	2 × 2/14 × 14	ND
Banha	2 × 2/30 × 25	
Sanyak	3 × 3/32 × 28	
Histamine	3 × 3/26 × 18	

ND. Not done.

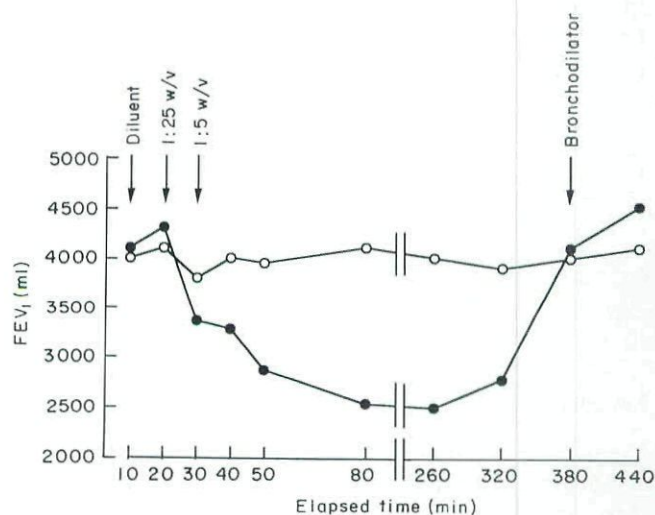
**Fig. 1.** The result of bronchoprovocation test with Sanyak extracts.

symptoms were aggravated after handling Sanyak and Banha, and improved when he was on vacation. On admission, physical examination showed clear breathing without wheezing.

A skin-prick test demonstrated immediate positive responses to cat fur, grass pollens, and Sanyak and Banha extract. Radioallergosorbent test (RAST) revealed positive responses as shown in Table 1. Total IgE level by PRIST was 1182 IU/ml. The total eosinophil count was 734/mm³. A chest X-ray revealed no abnormal findings.

Preparation of Sanyak and Banha extracts

The roots of Sanyak and Banha were obtained from the patient's pharmacy. The roots were cut into small pieces

**Fig. 2.** The result of bronchoprovocation test with Banha extracts.

and were extracted into phosphate-buffered saline (PBS, pH 7.5), 1:5 w/v, 4°C for 48 h, followed by centrifugation at 10 000 g at 4°C for 1 h. The supernatants were dialysed against 0.4% phenolized saline and were used for the bronchoprovocation tests at a concentration of 1:5 w/v. The protein contents of these solutions were 210 µg/ml and 70 µg/ml for the Sanyak and Banha extracts, respectively. For skin-prick test, the 1:5 w/v extracts were mixed with an equal amount of sterile glycerine. Some of the supernatant was dialyzed (cuf-off molecular weight was 6000 Da) against 4 l of distilled water at 4°C for 48 h, and lyophilized at -70°C. The protein content of the two lyophilized extracts were measured by the Lowry method [8]. One milligram Sanyak extract was found to contain

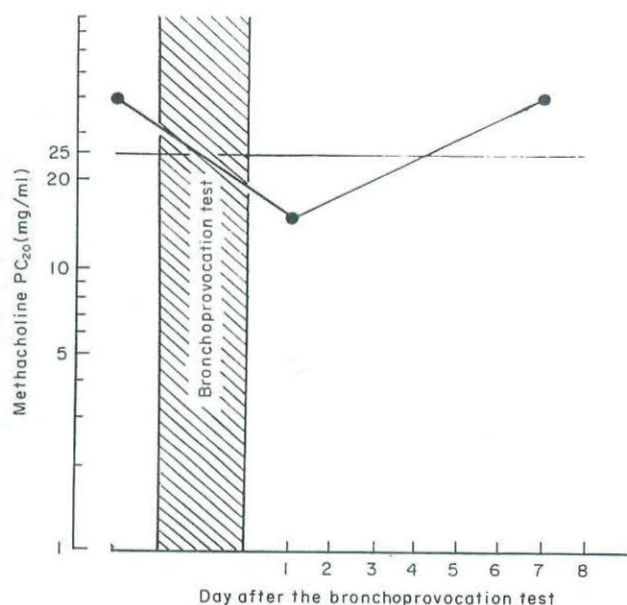


Fig. 3. The changes of methacholine PC₂₀ before and after the bronchoprovocation test with Sanyak extracts.

700 µg protein whereas 1 mg Banha extract contained 220 µg protein. These extracts were used as antigens for ELISA and SDS-PAGE.

Inhalation challenge test with methacholine

Airway responsiveness to methacholine was performed according to a method described previously [9]. In this study, methacholine challenges were performed one day before, and at 24 h and 7 days after the Sanyak-bronchoprovocation test.

Bronchoprovocation test with Sanyak and Banha

Bronchoprovocation tests were performed in a similar manner to other occupational asthma studies [10,11]. A phenolized saline solution was inhaled for 2 min from a nebulizer 646 (Devilbiss Co., Somerset, Pennsylvania, USA) connected to a compressed air source (5 l/min). The subject was asked to breathe the aerosol for 2 min with tidal breathing. The forced expiratory volume in one second (FEV₁) and peak expiratory flow rate (PEFR) were measured with a spirometer (Chest, Japan) before and 10 min after each inhalation. Then, the FEV₁ and PEFR were measured every 10 min during the first hour, and pulmonary function tests were performed every hour for 7 h after the challenge.

ELISA for specific IgE and IgG₄ antibodies to Sanyak and Banha extracts

The presence of specific IgE and IgG₄ antibodies to Sanyak and Banha was determined by ELISA. Microtitre

plates (Dynatech, USA) were first coated with 100 µl extracts of Sanyak (20 µg/ml) and Banha (100 µg/ml) dissolved in Carbonate buffer (pH 9.6). After incubation overnight at 4°C, each well was washed 3 times with 0.05% Tween phosphate buffered saline (PBS-T), and the remaining binding sites blocked by incubation with 250 µl of 10% new-born calf serum for 1 h at room temperature. The wells were then incubated for 2 h at room temperature with 100 µl of either the patient's serum (undiluted, 1:4 diluted, or 1:10 diluted) or control sera from five patients who showed negative skin-prick test responses to common inhaled allergens as well as Sanyak and Banha. After washing three times with PBS-T, 100 µl of 1:1000 w/v biotin-labelled goat anti-human IgE antibody (Sigma Chemical Co., USA) or biotin-labelled goat anti-IgG₄ antibody (Sigma Chemical Co., USA) were added to the wells and incubated for 2 h at room temperature. The wells were then washed three times with PBS-T and then incubated with 1:1000 w/v streptavidin-peroxidase (Sigma Chemical Co., USA) for 30 min before another washing step followed by incubation with 100 µl ABTS (2,2'-azido-ethylbenzthiazoline sulfonic acid in a citrate phosphate buffer) for 10 min at room temperature. The reaction was stopped by addition of 50 µl 2 M sodium azide and the absorbance was read at 410 nm by an automated microplate reader. All assays were performed in triplicate.

SDS-PAGE and immunoblotting

Sanyak (5 mg/ml) and Banha (20 mg/ml) were dissolved in a buffer (0.5 M tris-HCl, pH 6.8, 2.5 ml, glycerol 2.0 ml, 10% w/v SDS 4.0 ml, 0.1% bromophenol blue 0.5 ml and distilled water 1.0 ml) and boiled. Ten microlitres of standard markers (6-200 kDa) (Novex, San Diego, CA, USA) and antigen solutions were applied to a Novex precast tris-glycine gradient gel (4-20% acrylamide) for the separation of antigens. Electrophoresis was performed with a Novex Mini-cell for 90 min at 125 constant voltage. The gel was fixed and stained with Coomassie brilliant blue. For immunoblotting, the gel was soaked in transfer buffer (12 mM tris, 96 mM glycine in 20% methanol) for 10 min. Electroblotting was carried out for 90 min at 200 mA using a Novex western transfer apparatus. The blotted nitrocellulose membrane was then treated with a 0.5% tris-bovine serum albumin solution for 1 h to block non-specific protein binding. The patient's serum and control sera were diluted to 1:3 w/v with a TBS-T buffer (tris 10 mM, NaCl 150 mM, 0.05% Tween-80). The membrane was then incubated with the patient's and control sera for 2 h at room temperature. It was then washed with 0.1% triton in TBS-T for 10 min, and followed by washes with 0.5% triton in TBS-T for 10

Table 2. Specific IgE binding to Sanyak and Banha extracts in sera of the patient and control according to serum dilutions

Absorbance value Serum dilution	Sanyak patient	Control	Banha patient	Control
Undiluted	0.62	0.01*	0.01	0.00*
1:4 w/v	0.59	0.01*	0.02	0.01*
1:10 w/v	0.45	ND	0.00	ND
1:40 w/v	0.07	ND	ND	ND

ND. Not done.

* The absorbance values of control sera were mean values of five negative controls. Their individual values for Sanyak were 0.01, 0.01, 0.01, 0.005, 0.005 in undiluted serum and 0.01, 0, 0, 0, 0.005 in 1:4 w/v diluted serum. Those for Banha were 0, 0, 0, 0.005, 0.1 in undiluted serum and 0.005, 0.01, 0.01, 0.005, 0.01 in 1:4 w/v diluted serum.

Table 3. Specific IgG₄ antibody to Sanyak and Banha extracts in sera of the patient and control according to serum dilutions

Absorbance value Serum dilution	Sanyak patient	Control	Banha patient	Control
Undiluted	1.07	0.06*	0.02	0.01*
1:4 w/v	1.12	0.01*	0.02	0.01*
1:10 w/v	0.89	ND	0.00	ND
1:40 w/v	0.14	ND	0.00	ND

ND. Not done.

* The absorbance values of control sera were mean values of 5 negative controls. Their individual values for Sanyak were 0.07, 0.05, 0.06, 0.07, 0.04 in undiluted serum and 0.01, 0.01, 0.005, 0.01, 0.01 in 1:4 w/v diluted serum. Those for Banha were 0.005, 0.01, 0.01, 0.01, 0.01 and 0.01, 0.01, 0.01, 0.005, 0.01 in 1:4 w/v diluted serum.

min, and 0.5% NaCl in TBS-T for further 10 min each. Alkaline phosphatase-conjugated anti-human IgE antibody (Serotec #G 3918, UK) was diluted to 1:200 with TBS-T and used as a second antibody solution. The membrane was then incubated with the second antibody for 1 h at room temperature and washed again as above. A substrate solution (NBT/BCIP kit, Pierce #34041X, USA) was applied to the membrane until the positive bands appeared. After the reaction, the membrane was washed with distilled water and observed.

Results

Bronchoprovocation test with Sanyak and Banha

As shown in Fig. 1, there was no significant change after inhalation of 1:25 w/v Sanyak extracts, but a dual asthmatic reaction was noted after inhalation of 1:5 w/v

extracts. Figure 2 shows the result of the bronchoprovocation test using the Banha extracts. An early asthmatic reaction was observed after the inhalation of 1:5 w/v Banha extracts.

Inhalation test with methacholine

As shown in Fig. 3, the initial methacholine bronchial challenge test was negative. However, methacholine bronchial challenge test at 24 h revealed an increase in bronchial hyperresponsiveness to methacholine (PC₂₀: 15.0 mg/ml but this returned to normal bronchial responsiveness by the seventh day after the bronchoprovocation test.

Detection of specific IgE and IgG₄ antibodies to Sanyak and Banha extracts

Table 2 shows the specific IgE binding to Sanyak and Banha extracts in the patient's serum and control sera.

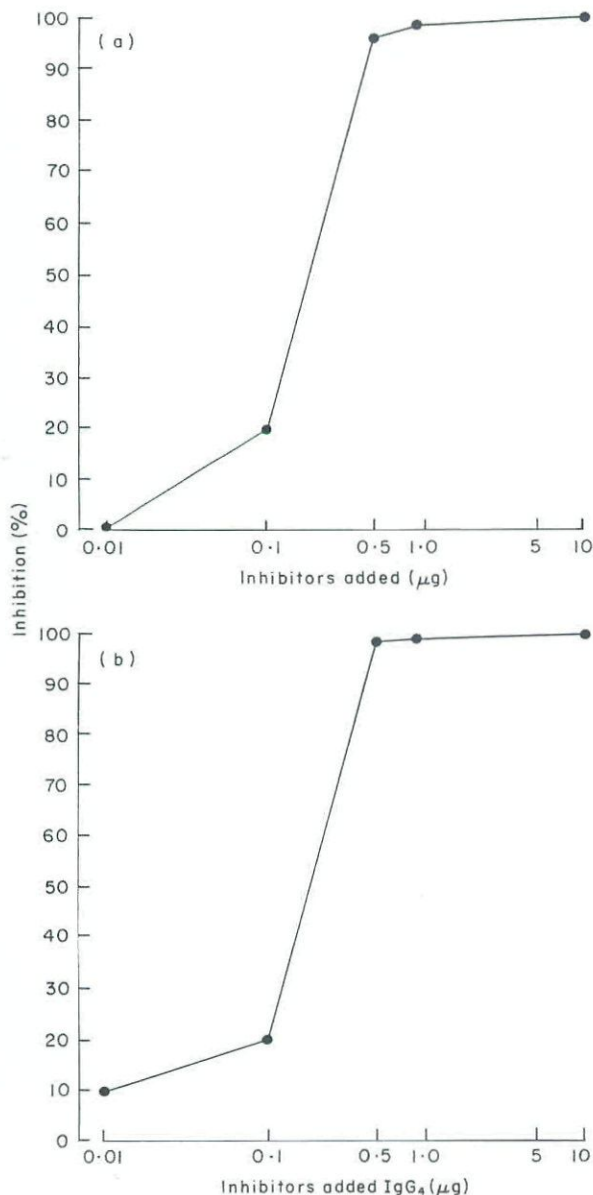


Fig. 4. Sanyak-ELISA inhibition test with addition of Sanyak extracts: (a) IgE-ELISA inhibition test; (b) IgG4-ELISA inhibition test.

The absorbance of control sera was mean from those of five control patients who showed all negative results on skin-prick test with 50 common inhalant allergens including two herb materials. Highly specific IgE binding to Sanyak extracts was noted in the various dilutions of the patient's serum in comparison to those of the controls. In the case of Banha, little binding was noted in both the patient's serum and control sera. Table 3 shows the specific IgG4 binding to Sanyak and Banha. Highly specific IgG4 binding was noted in the various dilutions of the patient's serum, in comparison with the negative

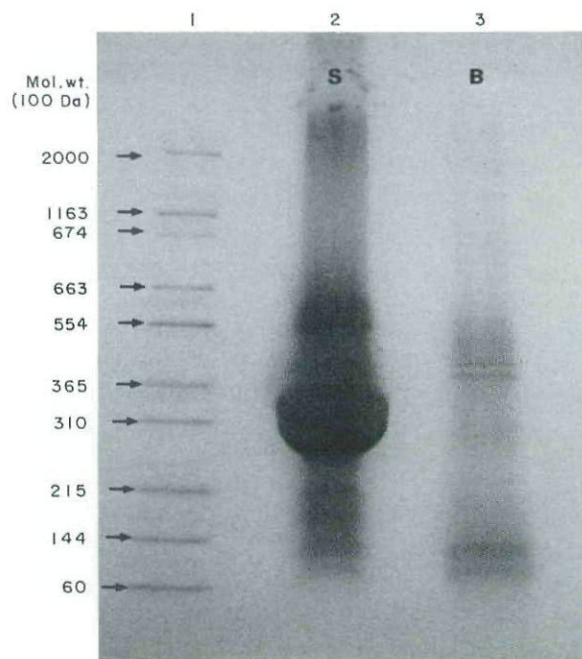


Fig. 5. Gradient SDS-PAGE (4–20%) of Sanyak (S) and Banha (B) extracts.

control's. In the case of Banha, little binding was noted in both the patient's serum and control sera. Figure 4 reveals the Sanyak ELISA inhibition (Fig. 4a: IgE ELISA inhibition test, Fig. 4b: IgG4 ELISA inhibition test) results with addition of the Sanyak extracts. Significant inhibition was noted with serial addition of Sanyak extracts in both ELISA inhibition tests.

Gel electrophoresis

In order to understand the protein components of Sanyak and Banha extracts, the extracts were analysed by a 4–20% gradient SDS-PAGE. Figure 5 reveals the resolution of the Sanyak extracts into more than 20 bands ranging from 6 to 200 kDa and resolution of the Banha extracts into more than 10 bands ranging from 10 000 to 90 000 Da.

Immunoblot analysis

Figure 6 demonstrates the binding of specific IgE in patient's (Fig. 6a) and control sera (Fig. 6b) on a blotted nitrocellulose membrane. With Sanyak extracts, there were three bands with molecular weights of 29 000, 57 000, 63 000 Da, bound to IgE antibody. With Banha extracts, no IgE binding component was noted.

Discussion

This study reveals that two commonly used herb materials, Sanyak and Banha, can induce occupational asthma

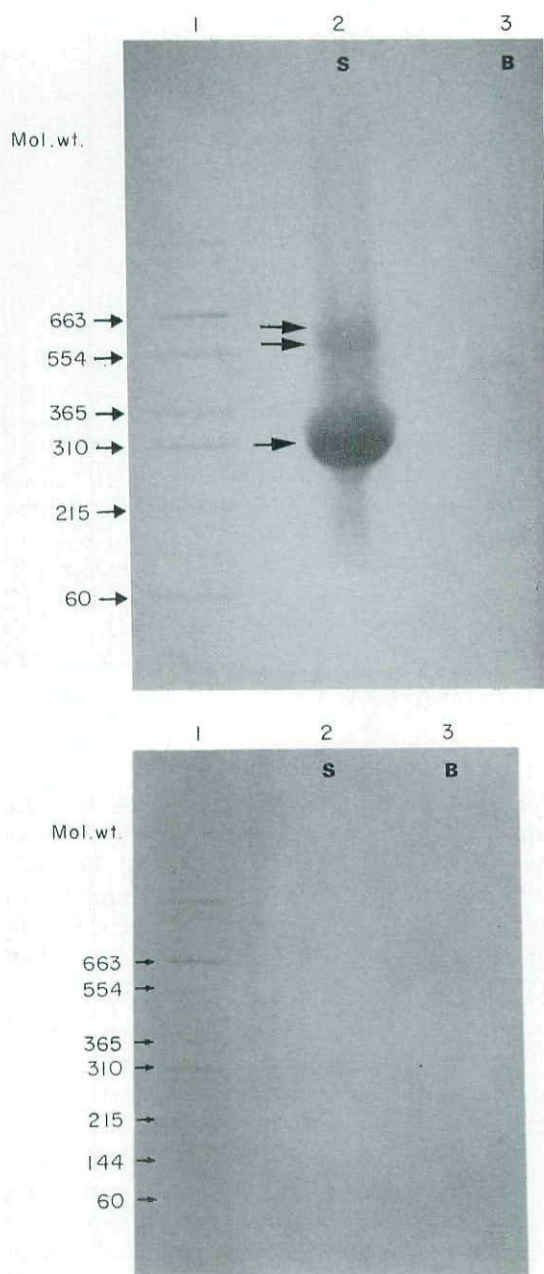


Fig. 6. Immunoblotting of Sanyak (S) and Banha (B) extracts and patient's serum (a) and control sera (b). Three IgE binding components were noted with Sanyak extracts (arrows).

in an exposed worker. This result was confirmed by bronchoprovocation tests. The patient also has a clinical presentation of occupational asthma without bronchial hyperresponsiveness to methacholine as other investigators have reported [13–15]. His bronchial hyperresponsiveness to methacholine developed at 24 h after the Sanyak bronchoprovocation test, and he had recovered by the seventh day with avoidance. Therefore, this study

suggests that careful inquiry about a patient's respiratory symptoms in the workplace is needed to identify occupational asthma patients. A negative methacholine challenge result alone does not necessarily preclude occupational asthma.

The pathogenetic mechanism of occupational asthma caused by Sanyak may be an IgE-mediated reaction, since the skin-prick tests with Sanyak showed a positive response to the Sanyak extracts. Furthermore, its specific IgE antibody was detected by ELISA. Also, the ELISA inhibition result suggested specificity of IgE binding to the Sanyak antigen. The immunoblotting study also showed three IgE binding components. When the bronchial challenge tests with these two herb materials were performed in two house dust mite-sensitive asthmatic patients, they showed negative responses. These results suggest that Sanyak can induce IgE-mediated bronchoconstriction in an exposed patient. Further investigations are needed to clarify the significance of specific IgG4 antibody in occupational asthma.

The mechanism of bronchoconstriction by Banha in this patient is unknown. Our results demonstrate that there is no serum-specific IgE to Banha in both ELISA tests in spite of a positive response on the skin-prick test studies. There is no serum specific IgG4 binding to Banha. The protein content of lyophilized extracts determined by the Lowry method was 700 $\mu\text{g}/\text{ml}$ in Sanyak and 220 $\mu\text{g}/\text{ml}$ in Banha extracts. For the SDS-PAGE, 5 mg/ml of Sanyak and 20 mg/ml of Banha were applied, which was enough to give a good resolution. As there was little binding to Banha in immunoblotting and ELISA, the possibility of IgE mediated reaction to Banha in this patient is very low. Further studies are needed to investigate the pathogenetic mechanism of Banha extracts.

There have been several reports that atopy plays an important role in the development of IgE sensitization to occupational allergens with high molecular weight [16–18]. The Sanyak extract had 700 μg of protein per 1 mg of extract. This antigen must be of macromolecular size because compounds with a molecular weight of less than 6000 to 8000 Da were excluded by the dialysis membrane we used. The patient has atopy and showed positive responses to grass pollens and cat fur. He said he had never experienced any rhinitis or respiratory problems before being employed at his workplace. There is little possibility of crossreactivity between grass pollens and Sanyak and Banha extracts because grasses are not taxonomically closely related to the latter plants. Also the taxonomical relationship is not close between Sanyak and Banha.

In conclusion, this study demonstrates that two herb materials, Sanyak and Banha, can induce occupational

asthma in an exposed patient. In the case of Sanyak-induced occupational asthma, it is possible that an IgE mediated immunological mechanism may be involved.

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