Dermatophagoides Farinae, an Important Allergenic Substance in Buckwheat-Husk Pillows

Chein Soo Hong, Hae Sim Park and Seung Heon Oh

Inhalation of buckwheat flour as well as ingestion of buckwheat foods induces bronchial asthma. The buckwheat flour attached to the husks used as bed pillow filling can provoke bronchial asthma in patients sensitized to buckwheat. In Korea one third of the pillows contain buckwheat husk (BH). Recently the authors examined three asthmatics who presented symptoms when exposed to house dust extract and BH-pillow extract. They had no history of asthma attack following ingestion of buckwheat foods. The possibility of mites or mite like substances in the BH-pillow extract was evaluated. There was a good correlation of skin test results between Dermatophagoides farinae (D. farinae) and BH-pillow extract. The concentrated extract of BH-pillow displayed inhibition of D. farinae radioallergosorbent test (RAST) and a dose dependent pattern on RAST inhibition. The lyophilized extract of BH-clean did not show any constant feature in D. farinae RAST inhibition test. Furthermore, mites of Dermatophagoides species were discovered in the contents of BH-pillows. Ten of 40 cases who tested positive to the skin test for the extract of BH-pillow manifested weakly positive to buckwheat RAST. None of them presented a previous history of buckwheat food allergy. Also we evaluated the possibility of cross-allergenicity between buckwheat and its husk.

Using these results the authors concluded that there were some amounts of allergenic substance from house dust mites in the contents of BH-pillows and that the dust from these could provoke or aggravate the symptoms of asthmatics who were sensitive to house dust mites.

Key Words: Dermatophagoides farinae, buckwheat husk pillow, bronchial asthma

Allergic reactions to buckwheat have been reviewed by many previous investigators since 1909 when first reported Smith that hypersensitivity manifestations such as asthmatic attacks, urticarial eruptions, gastrointestinal disorders, nasal symptoms or conjunctivitis were occasionally seen after ingesting or inhaling buckwheat or buckwheat flour.

Occupational allergic reactions to buckwheat were reported in bakers (Blumstein 1935; Ordman 1947) and in employees of buckwheat-noodle shops (Nakamura 1972; Nakamura et al. 1975). Most patients with occupational buckwheat allogene also exhibited hypersensitivity reactions after ingestion of buckwheat foods (Nakamura 1972; Nakamura et al. 1975). Matsuura et al. (1964) reported several cases whose asthma developed due to the buckwheat flour attached to buckwheat husks in pillows.

Buckwheat allergy has been reported in Korea. (Kang and Min 1984). Jang et al. (1985) reported a case of bronchial asthma induced by house dust and the buckwheat husk extract of pillows (BH-pillow, case 1 in Table 1).

Traditionally, many Koreans have used buckwheat husks as content of their pillows (Table 2). Recently we reviewed two more cases of bronchial asthma induced by BH-pillow extract (Table 1). It was interesting to note that each case exhibited strong skin reactions to house dust-dust mites, that the symptoms were also triggered by house dust (Torii Comp: Japan), and that there were no allergic manifestations following ingestion of buckwheat foods.

Therefore, we suspect that the substance in the contents of the BH-pillow which could induce bronchial asthma is dust mites.

MATERIALS AND METHODS

Patients and Sera

One hundred sixth seven patients with allergic
Table 1. Summary of clinical and laboratory data of patients challenged with buckwheat husk pillow extract

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/Age</th>
<th>PC20M* (mg/ml)</th>
<th>IgE (IU/ml)</th>
<th>Skin prick test1)</th>
<th>RAST</th>
<th>Buckwheat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D. farinae</td>
<td>BH-pillow**</td>
<td>D. farinae</td>
</tr>
<tr>
<td>1</td>
<td>F/30</td>
<td>1.1</td>
<td>588</td>
<td>13 × 17</td>
<td>3 × 3</td>
<td>Class 4</td>
</tr>
<tr>
<td>2</td>
<td>M/27</td>
<td>0.25</td>
<td>1000</td>
<td>9 × 17</td>
<td>6 × 6</td>
<td>Class 4</td>
</tr>
<tr>
<td>3</td>
<td>F/30</td>
<td>0.72</td>
<td>267</td>
<td>13 × 20</td>
<td>3 × 4</td>
<td>Class 2</td>
</tr>
</tbody>
</table>

BPT***

<table>
<thead>
<tr>
<th>Case</th>
<th>Housedust</th>
<th>BH-pillow</th>
<th>Use of BH-pillow</th>
<th>Allergy Reaction after ingestion of buckwheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 : 50</td>
<td>1 : 10</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dual</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 : 10</td>
<td>1 : 10</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Dual</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 : 10</td>
<td>1 : 10</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Early</td>
<td>Early(±)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* PC20M : PC20 of methacholine for bronchial sensitivity

** BH-pillow : Buckwheat husk pillow; skin test solution of BH-pillow: 1:20 w/v of 50% glycerine-0.4% phenol saline extract of buckwheat used as pillow content

*** BPT : Bronchoprovocation test

1) Wheal size (mm) 15 minute after prick test

2) % fall of FEV₁ was 13% at 30 minutes after last trial of 1:10 w/v of extract of BH-pillow

Table 2. The contents of pillows used by Korean allergic patients

<table>
<thead>
<tr>
<th></th>
<th>Negative skin test to D. farinae (n=221)</th>
<th>Positive skin test* to D. farinae (n=297)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buckwheat husk</td>
<td>29.0%</td>
<td>31.6%</td>
<td>30.5%</td>
</tr>
<tr>
<td>Rice husk</td>
<td>26.2</td>
<td>20.9</td>
<td>23.2</td>
</tr>
<tr>
<td>Sponge</td>
<td>24.0</td>
<td>25.6</td>
<td>24.9</td>
</tr>
<tr>
<td>Feather</td>
<td>11.3</td>
<td>10.8</td>
<td>11.0</td>
</tr>
<tr>
<td>Other</td>
<td>9.5</td>
<td>11.1</td>
<td>10.4</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* Positive skin test: >21mm of erythema size 15 minutes after skin prick test

rhinitis and/or bronchial asthma who visited the Allergy Clinic, Department of Internal Medicine, Yonsei University College of Medicine were tested with skin prick tests of house dust, Dermatophagoides farinae (Torii Comp., Japan) and the extract of the buckwheat husks from pillows which was made in our laboratory. The sera of all patients were stored at -20°C until needed for serologic examinations.

Sera of patients with RAST class 4 reaction to D. farinae (d2) and a negative skin test to BH-pillow extract were pooled for RAST inhibition tests. Sera of patients with negative results to skin prick tests of 50 inhalant allergens were also pooled for the negative control.

Buckwheat husk pillow extract

Five pillows containing buckwheat husks were collected from patients with strong skin reactions to house dust and dust mites. Equal parts of buckwheat husk from the five pillows were mixed, defatted with ethylether and dried. The buckwheat husks were suspended in 1:10 w/v of modified Coca's solution (Phillips, 1967: NaCl 9 g, phenol crystal 4 g, sodium

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bicarbonate 2.5 g in 1000 ml of distilled water) for 72 hours at room temperature. Following centrifugation, a 5 ml aliquot of the supernatant was dialysed against 2000 ml of 0.4% phenolized saline overnight in a cold room (Geissler et al., 1986; Arlian et al., 1987). After sterilization by ultrafiltration, the extract was mixed with an equal volume of autoclaved glycerine. This extract (1:20 w/v of the BH pillow) was used for skin test (BH pillow extract).

Concentrated extract of BH-pillow

A 1:10 w/v extract of BH-pillow in modified Coca's solution was placed in a visking tube and reduced in volume until it was approximately 15 times more concentrated than initial. The concentrated of BH-pillow extract was dialysed against phosphate buffered saline (PBS, pH 7.2) overnight at a temperature of 4°C.

Lyophilization of concentrated BH-pillow extract

Following dialysis against PBS, the concentrated extract was mixed with an equal volume of saturated ammonium sulfate solution. After centrifugation, the precipitate was dissolved in distilled water, dialysed against distilled water and lyophilized (lyophilized crude BH-pillow extract).

Lyophilized crude extract of buckwheat husk not used as pillows

Buckwheat husks not used as pillow contents were bought at a market. After defatting with ethylether the husks were suspended in modified Coca's solution (1:10 w/v) for 72 hours. The lyophilization procedure was as above (lyophilized crude BH-clean extract).

Lyophilized crude extract of Dermatophagoides farinae

Lyophilized crude extracts of D. farinae were kindly supplied by the Torii Comp. Japan.

Radioallergosorbent test (RAST) and RAST inhibition test

Phardebas RAST kits (Phamacia, Sweden) were used for RAST of D. farinae in samples treated with the diluted d5-sera-pool with a diluting solution of paper-radio-immunosorbent test kit (PRIST, Pharmacia, Sweden), concentrated BH-pillow extract, and 1 mg/ml of D. farinae for 1 hour at room temperature. Discs of D. farinae were incubated with the treated samples for 6 hours at room temperature. Anti-IgE tracers from the Phardebas RAST kit were incubated for 18 hours at room temperature.

RAST inhibition tests to D. farinae were performed. Discs of D. farinae from the Phardebas RAST kit were incubated with 100 µl serum for 6 hours at room temperature. The serum samples were combined with an equal volume of 1:40 diluted d5-sera pool and several concentrations of crude lyophilized extract of BH-pillow, BH-clean and D. farinae in PBS (pH 7.2) respectively for 1 hour at room temperature. All samples were examined simultaneously and in duplicate. Calculation of % inhibition as follows.

\[
% \text{ inhibition} = \frac{(\text{cpm of 1:40 d5-sera pool}) - (\text{cpm of samples})}{(\text{cpm of 1:40 d5-sera pool})} \times 100
\]

RAST with buckwheat was performed on the stored sera of patients exhibiting positive skin prick tests to the BH-pillow extract.

Dot-radioimmunoassay of buckwheat husk

A modification of the dot-immunobinding assay (Derer et al., 1984; Hong et al. 1985) was performed on the buckwheat husk.

The lyophilized BH-clean extract was dissolved in 1% KOH and adjusted to a final concentration of 20 mg/ml. One ul of solution was dotted on nitrocellulose filter paper (Millipore Type HA H5D 75990A). After drying for 2 hours at room temperature the dotted discs were washed with tris buffered saline (50 mM tris/NaOH in 200 mM NaCl pH 7.4, TBS). Following incubation with 10% newborn calf serum in TBS, the dotted discs were then combined at room temperature with 50 µl per sample of serum with positive skin prick tests. After washing 3 times with TBS, 50 µl of RAST tracer was added to each and incubated for 18 hours at room temperature. The discs were washed with TBS three times, and then counted in a gamma-ray counter.

Discovery and identification of mites in dusts of buckwheat husk

Ninety five percent lactic acid was poured into a petridish onto the dust collected by sieving BH-pillow and BH-clean. Using a dissecting microscope mites were found, picked up with a 26 gauge needle, and placed in a solution of lactophenol for 48-72 hours at room temperature. The mites were mounted on slides with Berlese mounting medium (Hoyer’s method: distilled water 25 ml, Chloral hydrate 100 gm, Glycerine 10 gm, Gum arabic 15 gm, cited in Cho and Lee 1981), and then identified.
Table 3. Results of skin prick tests for the extracts of Dermatophagoides farinae and buckwheat husk pillow

<table>
<thead>
<tr>
<th></th>
<th>D. farinae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>BH-pillow</td>
<td>62**</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>&gt;10 mm of</td>
<td>0</td>
</tr>
<tr>
<td>wheal size</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
</tr>
</tbody>
</table>

* + skin test: >21 mm of erythema size 15 minutes after skin prick test
** Number of the tested patients
D. farinae: Prick test solution from Torii comp.
BH-pillow: The extract of buckwheat husk pillow made as 1:20 w/v in glycerine 0.4% phenolized saline

RESULTS

Forty five of 167 cases exhibited a positive skin test to BH-pillow extract (Table 3). Patients with stronger reactions to D. farinae also presented more frequent and stronger reactions to the extract of BH-pillow.

Fig. 1 shows the results of RAST of D. farinae after incubation with diluted serum samples from the strongly positive sera pool and several kinds of solutions for one hour at room temperature. After incubation with the concentrated extract of BH-pillow, RAST of D. farinae exhibited significantly lower cpm in all sera dilution samples than those incubated with dilute solutions of PRIST (B in Fig. 1).

When larger amounts of concentrated BH-pillow extract were incubated with the diluted sera pool, RAST of D. farinae of these samples had lower counts than those of the 1:1 mixture (C in Fig. 1). But the fewest cpm were obtained from the samples treated with 1 mg/ml of crude extract of D. farinae. These results suggested that there was a certain amount of mite substance cross-reacting with D. farinae in the concentrated of BH-pillow extract.

To evaluate the presence of mites or a mite-like substance in the buckwheat husks not used in pillows, RAST inhibition tests were done with the crude extract of BH-pillow, BH-clean and D. farinae. Table 4 shows the percent inhibition of D. farinae RAST by the lyophilized crude extracts which were incubated with 1:40 diluted samples from the strongly positive sera pool. Definite inhibition of D. farinae RAST was noted in the 10 mg/ml concentration of the lyophilized crude extract of BH-pillow. The percent inhibition of

10 mg/ml of lyophilized crude extract of BH-pillow was nearly the same as that of the 0.001 mg/ml of lyophilized crude extract of D. farinae. The inhibition of D. farinae RAST with lyophilized crude extract of BH-pillow revealed a dose-dependent pattern. There was no constant inhibition of D. farinae RAST when reacted with the lyophilized crude extract of BH-clean.

Fig. 2 shows the result of RAST of buckwheat (RAST-f11) in patients with positive skin test to the BH-pillow extract. Ten cases out of 40 demonstrated a weak positive reaction to RAST-f11. The rate of RAST-f11 positivity was not correlated with the use of a buckwheat-husk pillow. None of cases which exhibited a positive RAST-f11 presented any allergic histories to foods made from buckwheat. The case with class 2 in RAST-f11 also had a high IgE level (over than 1000

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Table 4. Percent inhibition of D. farinae RAST by the lyophilized crude extracts

<table>
<thead>
<tr>
<th>Allergen concentration of incubated sample (mg/ml)</th>
<th>BH-pillow*</th>
<th>BH-clean**</th>
<th>D. farinae</th>
<th>N.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>24.7%</td>
<td>-9.6%</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>16.1</td>
<td>6.3</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>8.8</td>
<td>12.1</td>
<td>98.1%</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>-6.4</td>
<td>-9.4</td>
<td>89.3</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>-8.6</td>
<td>-4.0</td>
<td>64.6</td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>1.4</td>
<td>-4.5</td>
<td>28.6</td>
<td></td>
</tr>
</tbody>
</table>

* BH-pillow: Lyophilized crude extract of buckwheat husk used as pillows
** BH-clean: Lyophilized crude extract of buckwheat husk not used as pillow

Protein contents of extracts by Lowry method: 1 mg/ml of BH-pillow, BH-clean and D. farinae were 1.213, 1.417, and 0.685 mg/ml respectively.

Fig. 2. The result of RAST of buckwheat and skin test of the extract of buckwheat husk pillow according to use of buckwheat pillow.

Skin prick test of the extract of buckwheat pillow

Fig. 3. Relationship between radioimmuno-dot assay of buckwheat husk and radioallergosorbent test of buckwheat in patients with positive skin prick test of the extract of buckwheat husk pillow.

Fig. 4. Two Dermatophagoides farinae, a male and a female, of which were discovered in the dust from a buckwheat husk pillow.

The buckwheat husks in the pillow were dusty and fragmented, and thus a much larger amount of dust was obtained by sieving the BH-pillow rather than the BH-clean. Thirty-two mites were found in approximately 500 mg of dust from the BH-pillow. Twenty-seven were identified as D. farinae, and two as D. pteronyssinus. The remaining three mites were Dermatophagoides spp., which could not be identified. There were no mites in about 1.0 gm of dust sieved from BH-clean.

**DISCUSSION**

Buckwheat is one of the important allergenic foods. Buckwheat can trigger allergic symptoms following inhalation of the flour as well as ingestion of foods containing buckwheat. Most patients who exhibit allergic symptoms after inhaling buckwheat flour also develop allergic reactions after ingesting buckwheat foods. The patients who developed asthma from the buckwheat flour attached to buckwheat husk in pillows, reported by Matsumura et al. (1964), also developed asthma after eating buckwheat foods.
In Korea one third of the pillows contain buckwheat husks, but only a few cases of buckwheat allergy have been reported (Kang, 1984). Among inhalant allergens, the most important to Korean bronchial asthmatics is house dust-dust mites (Kang, 1973; Whang et al., 1973; Cho et al., 1981; Hong et al., 1982; Kim et al., 1983; Hong et al., 1987).

We reviewed bronchial asthmatics whose symptoms were induced when challenged by house dust extract and the extract of buckwheat husks used in pillows. They did not exhibit any allergic reactions after ingestion of buckwheat foods. Therefore we suggest that there is a house dust mite substance in the pillows which contain buckwheat husks.

Table 3 shows significant correlations between skin reactivity to Dermatophagoides farinae and to buckwheat husk pillow extract. Patients with a strong reaction to D. farinae more frequently exhibited a positive reaction to the extract of buckwheat husk pillows.

The concentrated husk pillow extract manifested some inhibition of RAST to D. farinae in a dose-dependent pattern (Fig. 1). However, the extract of clean buckwheat husk displayed no RAST inhibition to D. farinae (Table 4).

These results suggest that the extract obtained from bed pillows contains a small amount of allergenic substance which cross-reacts with D. farinae.

This speculation was confirmed by the discovery of mites in the dust sieved from the pillows. It was also noteworthy that all the mites discovered in the pillow dust were Dermatophagoides species.

Ten of the 40 cases with skin reactions to BH-pillow manifested a weakly positive reaction to buckwheat RAST (Nine cases, class 1; one, class 2). Even though there were 7 RAST positive cases in the 12 case group of 4+ skin reaction to BH-pillow, there did not appear to be a relationship between skin reactivity and RAST class. Also there were no cases with high RAST class to buckwheat.

The most important and definitive diagnostic method in food allergy is the double-blind oral challenge test (Bernstein et al. 1982; Atkins et al. 1985). Most patients with occupational buckwheat allergose
manifested allergic reactions after oral challenges with buckwheat (Nakamura et al. 1975). Five of 9 cases, who experienced asthma attacks brought about by the buckwheat flour attached to buckwheat husks in pillows, also developed bronchial asthma after oral challenge with buckwheat foods (Matsumura et al. 1969). In this study the patients who were RAST positive to buckwheat and demonstrated a positive skin test to the BH-pillow extract, did not experience any allergic reactions after ingesting buckwheat foods.

The allergenicity of the buckwheat husk is still unknown. Based on the dot RIA of buckwheat husk there would be a cross-antigenicity between buckwheat and buckwheat husk. Two of 30 buckwheat RAST negative cases and 6 of 10 buckwheat RAST positive cases were positive in dot-RIA of buckwheat husk with a cut-off point of a 2 fold more binding radioactivity than the negative control samples. Even though we can not rule it out completely, the possibility exists that the buckwheat husk itself is a cause of asthma and will be examined in subsequent experiments.

Korean houses employ an Ondol heating system. The floor of the room is very dry and smooth in general, not carpeted. The primary offending inhalant allergen in respiratory allergy is the house dust mites, members of Dermatophagoides species (Cho, 1980). The dusty areas around and under furniture and household goods made of organic materials, such as buckwheat husk pillows make good breeding areas for house dust mites.

In summary we can conclude that there are small amounts of allergenic substances including house dust mites in buckwheat husk pillows, and that these may provoke or aggravate the symptoms of asthmatics who are sensitive to house dust mites.

ACKNOWLEDGEMENT

We would like to thank Kyung Soo Hahn Ph.D. for the measurement of protein amounts in lyophilized mite extracts, BH-pillow and BH-clean by Lowry method.

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