Oral Desensitization Therapy Using Hypoallergenic Wheat for Children with Wheat Allergy

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ABSTRACT

The oral desensitization using hypoallergenic wheat was performed for twenty children with a history of wheat allergy. The enrolled subjects were divided into three groups: children with severe wheat allergy, group S; children with mild wheat allergy, group M; children receiving no desensitization, group C. The oral desensitization was performed with increasing doses starting at 25 mg (group S) or 0.4g (group M) using hypoallergenic wheat. Six out of eight children in group S and five of seven children in group M achieved the daily intake dose of wheat product, whereas only one of five children did in group C. Hypoallergenic wheat is useful for inducing food tolerance for children with a wheat-immediate type allergy.

INTRODUCTION

Food allergy plays an important pathogenic role in up to 40% of infants and children [1,2]. Strategies for the prevention of allergy have been proposed, including the use of products with extensively reduced allergenicity. Products designed to have reduced allergenicity have also been proposed and marketed in Japan as hypoallergenic formulas: "Fine Rice" is a product that was first developed to produce a hypoallergenic rice preparation[3, 4].

In addition to rice, cereal allergy is another major problem in many countries where cereals have habitually been consumed. In fact, in Japan, wheat is the third commonest food causing allergy in children following egg and cow's milk [5]. Although

allergies to egg and cow's milk can be tolerated by elimination, wheat allergy is thought to be untolerated by elimination. Food allergy is believed to result from a breakdown of normal oral tolerance induction [6]. While an immunotherapy by injection has been proved unsafe in food allergy [7], some investigators have had partial success with oral immunotherapy [8].

We have recently developed hypoallergenic wheat products [9-14], and have reported the safety and usefulness of the hypoallergenic wheat products in patients with atopic dermatitis (AD) having wheat allergy [15].

To investigate whether oral desensitization is able to induce food tolerance for children with a wheat-immediate type allergy. We performed an open controlled study of hypoallergenic wheat oral immunotherapy in children having wheat allergy with a history of immediate allergic reaction to wheat.

MATERIAL AND METHODS

Subjects

Subjects were twenty children who have severe IgE mediated wheat allergy(male/female: 13/7, age 4.1±2.3 years in mean ± SD). The characteristics of the subjects are shown in Table 1. Wheat allergy was defined as an episode of allergic reaction such as urticaria, wheezing, and/or vomiting when they ate a small amount of bread or pasta-like noodles, and a positive opened food challenge test using "udon" noodle and a positive food challenge test using wheat products. These allergic episodes were confirmed within a year prior to the study entry.

Our previous study reported that hypoallergenic cupcake(wheat) is safe for most

Table 1 *Characteristics of 20 Patients with Wheat-Allergy*

Patient No	Age	Sex	Clinical Symptom	RAST-IgE Wheat	ELISA-IgE Wheat	ELISA-IgE HW#	RAST-IgE Gliadin	RAST-IgG Gliadin
1	3	M	U	100	0.125	0.095	19	49.6
2	6	F	W	100	0.526	0.45	100	35.8
3	3	M	A	100	0.322	0.166	9.71	14.5
4	5	M	A	16	0.127	0.088	0.35	9.57
5	5	M	A	100	0.31	0.26	3.17	19.6
6	3	M	U	100	0.556	0.326	20.6	11.8
7	5	M	A	31.4	0.158	0.092	0.35	6.7
8	7	M	W	ND	0.498	0.386	87.2	20.2
9	10	M	W	100	0.34	0.026	0.35	9.4
10	1	F	U	2.24	0.009	0.013	0.35	5.16
11	2	F	U	10	0.031	0.021	0.35	20.2
12	1	F	U	30.5	0.087	0.008	1.81	10.3
13	3	F	U	94.5	0.274	0.047	7.97	18.8
14	3	F	U	3.45	0.006	0.001	0.35	10.4
15	6	M	W	ND	0.037	0.015	0.35	9.4
16	1	M	W	20	0.053	0.018	2.93	20.6
17	3	M	W	100	0.278	0.158	8.66	67.3
18	3	M	W	89.4	0.223	0.091	28.2	25.6
19	5	M	U	0.62	0.005	0.007	0.35	2.93
20	7	M	U	44.1	0.020	0.026	0.35	6.24

1-8: Sever group, 9-15: Mild group, 15-20: Control group
ND: not done U:urticaria W:wheezing A:anaphylaxy
F:female M:male HW:hypoallergenic wheat

patients with wheat allergy, if ELISA of the hypoallergenic wheat is less than 0.05(15). The enrolled children were divided into three groups, based on the value of ELISA of the hypoallergenic wheat: Group S, children allocated to the hypoallergic wheat ingestion demonstrating high levels (>0.05) of ELISA (enzyme linked imunosorbent) IgE value for hypoallergic wheat at the time of study entry; Group M, children allocated to the hypoallergic wheat ingestion demonstrating low levels (<0.05) of ELISA IgE value for hypoallergic wheat at the time of study entry; Group C, children allocated to no wheat ingestion.

Ethical approval was obtained through the Institutional Review Boards at Kansai Medical University, and written informed consent was obtained in accordandce with each institution's ethics guidelines for research in children.

Study protocol

Table 2 *Desensitization Protocol of Hypoallergenic Wheat*

Month of oral desensitization	Hypoallergenic wheat#	Wheat
0	25mg	
1	50mg	
2	100mg	
3	200mg	
4	400mg	
5	800mg	
6	1g	
7	2g	
8	4g	
9		0.5g
10		1g
11		2g
12		4g
13		8g
14		16g
15		32g
16		64g
17		100g

#: hypoallergenic cup cake was ingested starting 400mg of hypoallegenic wheat

Groups S: 25 mg of hypoallergenic wheat was initially given at an outpatient clinic or on admission. Intravenous doses of diphenhydramine and intramuscular epinephrine were ready at the bedside at all times.

Subjects were then observed for two hours, and if no evidence of allergic reaction was observed, they were discharged. Subjects began twice-weekly dose of 25 mg at home, which was continued for a month.

After a month, patients visited the outpatient clinic to ingest 50 mg, twice the initial dose. Table 2 shows a summary of the protocol schedule for the severe group. Finally, the enrolled children ingested 4 g of hypoallergenic cupcake.

Group M: 0.4 g of hypoallergenic cupcake was initially given. The subsequent schedule was the same as that of the group S.

Group C: No wheat product was given during the study enrollment (eight months). The children did not ingest any kind of wheat product during the period.

After the trial of hypoallergenic wheat, 0.5 g of wheat ("udon" noodle) was given in the clinic. If no allergic reaction was observed, they were discharged home and continued to ingest the same dose twice a week for a month. The dose continued to double each month until ingestion of 100 g was reached. If a severe allergic reaction occurred, the protocol was discontinued. If a mild reaction was observed, anti-histamine and beta-two stimulant inhalation was given. In the group C, after the observational period (no hypoallergenic wheat trial) the wheat-trial was performed in the same way as for the group S and M.

Blood samples: Venous blood samples were collected before and after the cupcake trial. In the group S, additional blood sampling was performed three months later since beginning of the cupcake trial. In the group C, blood sampling was also performed before and after the obser-

vational period.

Production of hypoallergenic flour: Hypoallergenic flour was prepared by the method previously described [15]. As wheat flour is made hypoallergenic by hydrolyzing peptide bonds near the essential residues of the epitope [12], actinase was selected for its production. Wheat flour was treated in a 6:10 mixture of water and flour with acinase at 37C for 1 hour. From ELISA data using serum samples from patients allergic to wheat flour, no allergenicity was detected with the hypoallergenic flour [13, 14]. As a result of this procedure, the allergenicity of wheat flour decreased to less than a tenth of the original value [14]. As the glutenin-type protein in bakers' yeast has a high structural similarity to the allergenic peptide of gluten [11], commercially available baking powder was used as a carbon dioxide generator. The hypoallergenic flour was mixed with liquid jelly, sugar, baking powder, hypoallergenic margarine, salt, surfactant and a small volume of vanilla essence, and portions of the mixture were poured into cups and steamed. One gram of hypoallergenic wheat flour was prepared from one gram of wheat flour. A single cupcake contains 1.74 grams of hypoallergenic flour.

Establishment of the method for intact flour, hypoallergenic flour and omega-5 gliadin: ELISA for intact flour and hypoallergenic flour was carried out by the method previously described [15]. RAST specific IgE for wheat, omega-5 gliadin, and RAST specific IgG for omega-5 gliadin were measured by using the Unicap 100 system (Pharmacia Diagnostic AB, Uppsala, Sweden).

Statistical analysis

Stastistical analysis was performed by using the Wilcoxon matched-pairs signed-ranks test and chi-square test with Yates correction in StatView J-5. A p-value of less than 0.05 was considered significant.

RESULTS

Success of the desensitization protocol:

In the group S, six of eight children (75%) achieved a daily intake of 100g of wheat products. Indeed, three of six children reached the daily dose without showing any symptoms. The other three children presented some symptoms, which started shortly after the ingestion of wheat and persisted for two hours. One of the eight children presented a severe allergic reaction at 50 mg of ingestion of hypoallergenic wheat, after which the schedule was discontinued. Another of the eight children was able to tolerate 35 g of "udon" noodles. Increasing the intake dose to 50 g resulted in wheezing after about 10 min.

In the group M, five of seven children (71.4%) also achieved the daily intake of 100g of wheat products without any symptoms. One of the seven children showed a severe allergic reaction at 1 g of ingestion of hypoallergenic wheat, including wheezing and urticaria, resulting in the discontinuation of the schedule. Another of these seven children was able to tolerate 15 g of "udon" noodles. Increasing the intake dose to 35 g resulted in an allergic reaction after about 30 min.

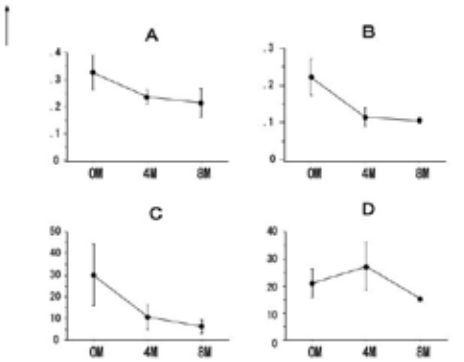
In the group C, after the observational period (average: 8 ± 0.83 months), wheat-ingestion was also attempted for the enrolled children. The schedule was discontinued for four of the five children because of severe allergic reactions. The remaining child was able to ingest the daily dose of 100g of wheat products.

Altogether, 13 of 15 children who ingested hypoallergenic wheat were desensitized, but only one child in the control children became able to ingest wheat. This result was significant ($P<0.05$) using chi-square test with Yates correction

Specific IgE levels for each wheat products and specific IgG concentration for gliadin (Fig. 1-3):

In the group S, the levels of ELISA IgE for wheat and hypoallergenic wheat gradually decreased during desensitization (Fig. 1A&1B), as did the RAST IgE for gliadin (Fig. 1C), but no significant difference was observed. The levels of RAST IgE for wheat and RAST IgG for gliadin (Fig. 1D)

Fig. 1. *Serial Changes in Serum Levels of IgE and IgG in the Group S.*



Blood was taken from the patient at the time of 0, 4 and 8 months (n=7): A, ELISA specific IgE level for wheat flour; B, ELISA specific IgE level for hypoallergenic wheat; C, RAST specific IgE level for omega-5 gliadin; D, RAST specific IgG for omega-5 gliadin.

did not decrease during desensitization.

In the group M, the ELISA IgE values for hypoallergenic wheat significantly decreased during the period (Fig. 2B, $P<0.05$), whereas other parameters tended to decrease without statistical significance.

In the group C (Fig.3), there was no significant difference in any parameters during the study period.

DISCUSSION

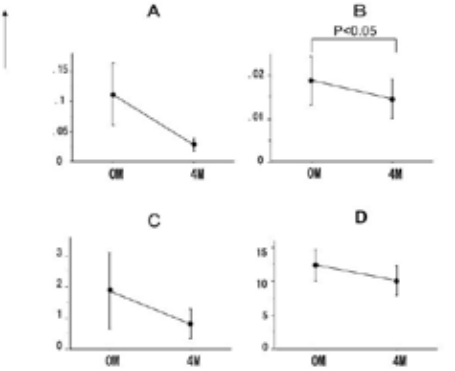
While children with food allergy may develop tolerance, it is believed that tolerance does not develop over the age of five. Recently, wheat allergy has increased in Japanese children since the custom of consuming cereal products was established. Wheat is present in a variety of daily foods in Japan. Many patients with wheat allergy have experienced tremendous problems as a result of eating wheat products. Though many studies in oral food desensitization have been reported [18, 19], no studies of oral desensitization for wheat allergy exist. Neither has there been any trial of oral food desensitization using hypoallergenic wheat. In this regard, our study is the first to try desensitization for wheat-allergic children by using hypoallergenic wheat, and moreover has been successful. Although it cannot be excluded that the patients may have tolerated during the study period, the study may be significant since four of five children in the control group could not ingest wheat products during the same period.

The mechanism of oral desensitization of food allergy is considered to be clonal depletion, clonal anergy, active suppression and production of blocking antibody (20). Low doses of antigen mostly induce active suppression, whereas high doses favor deletion and anergy [21]. According to one study [22], oral desensitization using modified fish antigens was successful for patients with fish allergy. Recent studies have demonstrated successful oral food desensitization by starting with a very small volume of cow's milk and egg powder [18, 19].

In the present study, we speculate that two factors, namely low dose of antigen loading and modified peptides, induce active suppression and produce a blocking antibody, resulting in oral tolerance.

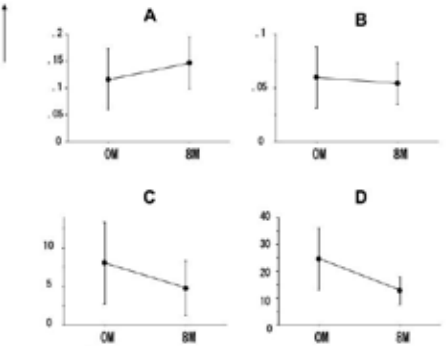
The peptides contained in hypoallergenic wheat are reduced to 10% of total peptides digested by actinase and cellulase. Recent studies show that omega-5 gliadin is a

Fig. 2. *Serial Changes in Serum Levels of IgE and IgG in the Group M.*



Blood was taken from the patient at the time of 0, 4 and 8 months (n=6): A, ELISA specific IgE level for wheat flour; B, ELISA specific IgE level for hypoallergenic wheat; C, RAST specific IgE level for omega-5 gliadin; D, RAST specific IgG for omega-5 gliadin.

***Fig. 3**. Serial Changes in Serum Levels of IgE and IgG in the Group C.*



Blood was taken from the patient at the time of 0, 4 and 8 months (n=5): A, ELISA specific IgE level for wheat flour; B, ELISA specific IgE level for hypoallergenic wheat; C, RAST specific IgE level for omega-5 gliadin; D, RAST specific IgG for omega-5 gliadin.

significant allergen in children with immediate allergic reactions to ingested wheat and in adults with food-dependent, exercise-induced anaphylaxy [23, 24]. We also found that omega-5 gliadin comprised a small amounts of total wheat products in hypoallergenic wheat (unpublished data). Our data showed that RAST IgE for gliadin decreased in association with the reduction of ELISA IgE for hypoallergenic wheat and wheat during the oral desensitization, although these decreases were not significant changes. Given the lack of any increase in the levels of RAST IgG for gliadin, it seems likely that the mechanism of oral tolerance is, at least in part, due to the active suppression of IgE antibody production.

In conclusion, our study is the first to show that hypoallergenic wheat is a useful food for inducing food tolerance of wheat by oral desensitization for children with wheat allergy. Further study will be needed to evaluate the starting dose and the frequency of ingestion of hypoallergenic wheat.

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