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Mass Trial of Hypoallergenic Rice (HRS-1) Produced by Enzymatic Digestion in Atopic Dermatitis with Suspected Rice Allergy

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The clinical usefulness of a hypoallergenic rice (HRS-1) which was produced by enzymatic decomposition of the constituent proteins of original rice was evaluated in a multicentre study in 44 subjects with recalcitrant atopic dermatitis (AD), who were suspected of having rice allergy. The subjects were fed for at least 4 weeks with HRS-1 instead of eliminating both regular rice and wheat from their daily diet. The extent of overall skin lesions was expressed by using the AD affected area and severity index (ADASI). A statistically significant decrease in ADASI was observed at the 2nd and the 4th week readings and at the end of the study. A provocation test with regular rice was carried out in 5 of 44 subjects following the HRS-1 therapy. An obvious increase in ADASI was found in all of these 5 cases just after this procedure. On final evaluation, 77% of the patients tested showed 'moderate' to 'remarkable' improvement, and 59% of the patients a 'moderate' to 'remarkable' reduction in use of the steroid ointment concomitantly used for the treatment. Finally, HRS-1 was evaluated as 'useful' or 'very useful' in 69% of the subjects. ***Key words: Severe atopic dermatitis; Rice allergy; Wheat allergy; Hypoallergenic food; Elimination diet.***

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INTRODUCTION

Recently, the severe type of atopic dermatitis (AD), which is not very well controlled by steroid ointment, has been noted to be on the increase in Japan. From a statistical analysis of the correlation between rice-RAST score and clinical severity in AD patients, Ikezawa et al. (1) have suggested a probable involvement of rice allergy in many severe cases of AD. In fact, Komatsu et al. (2) have reported that an elimination of rice from the diet in these cases resulted in varying degrees of improvement. Moreover, from the high correlation coefficient among RAST values for cereals, and the cross-inhibition in CAP-RAST and IgE-immunoblotting for rice/wheat, Tsubaki et al. (3) have revealed that there are partial cross-reactions between rice and wheat allergens.

Since rice and wheat are consumed as staple foodstuffs in Japan, it is very difficult to eliminate them from the daily diets. We therefore, developed a hypoallergenic rice (HRS-1) by enzymatic decomposition of the constituent proteins which are presumed to be the major allergens of rice (4). In a preliminary trial, HRS-1 has already been evaluated and found useful as a replacement food-stuff for the elimination of rice from the diet (5). In this paper, its clinical usefulness was examined in many AD patients who were suspected of rice allergy, in collaboration with 13 hospitals.

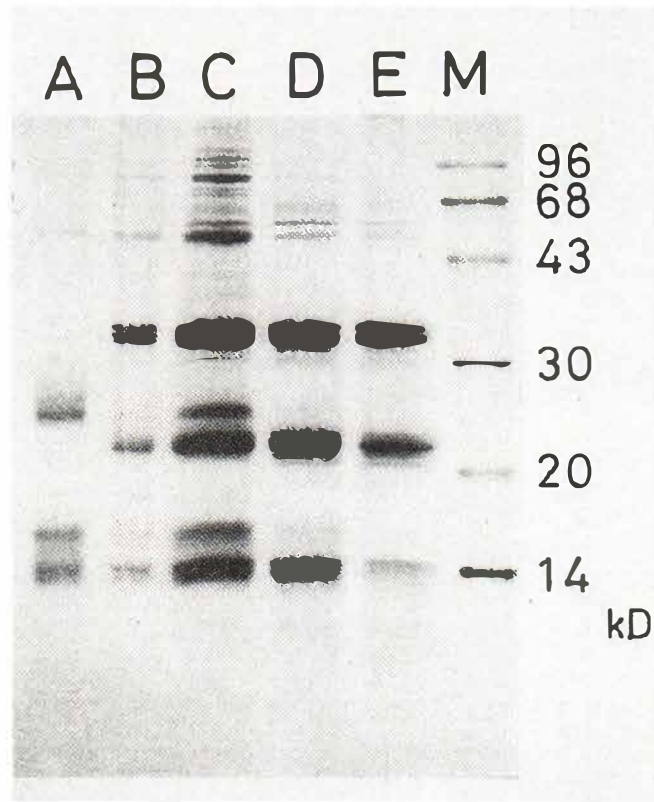

Fig. 1. SDS-PAGE of rice proteins. A: salt-soluble (globulin) fraction in original rice; B: alkali-soluble (glutelin) fraction in original rice; C: original rice; D: rice treated with actinase but not with surfactant under the degassing process; E: rice treated with actinase and surfactant under the degassing process (the HRS-1); M: marker proteins.

Abbreviations
AD: atopic dermatitis
ADASI: atopic dermatitis area and severity index
HRS-1: hypoallergenic rice of Shiseido-1
kD: kilo Daltons
PASI: psoriasis area and severity index
RAST: radio-allergo-sorbent test
SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

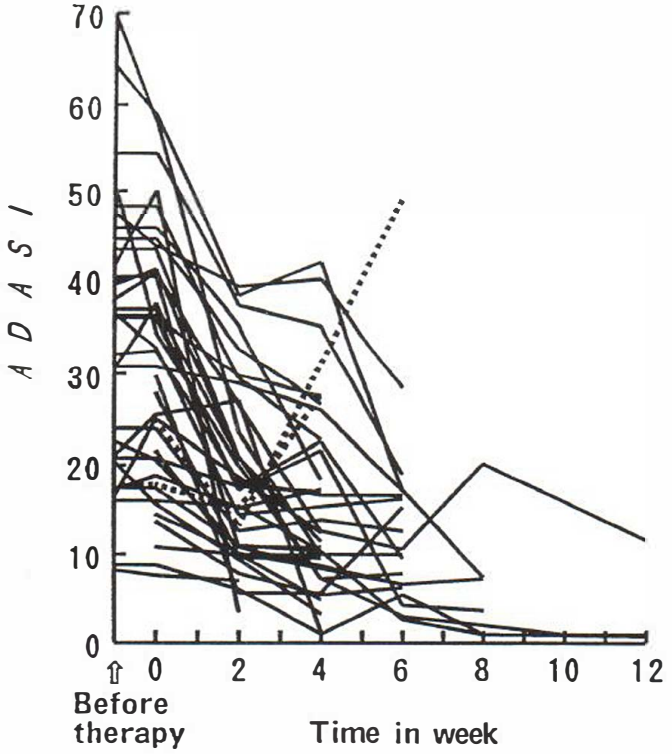

Fig. 2. Time course of each ADASI for the 39 subjects during the replacement therapy with HRS-1. A remarkable decrease in ADASI was observed in most cases (——), but obvious exacerbation was also observed, in 2 cases (-------).

MATERIALS AND METHODS

Hypoallergenic rice (HRS-1; Fine rice®)

HRS-1 was produced by treating of the newly harvested rice grains (*Oryza sativa L. japonica,* cultivar Koshihikari) with proteolytic enzyme (4). Protein analysis of the HRS-1 by SDS-PAGE revealed almost total disappearance of 16 kD, 25 kD and other high molecular bands (50 kD, 90 kD, etc.), corresponding to the globulin fractions extractable with 1 M NaCl from the original rice, as shown in Fig. 1 (4, 5). Especially the 16 kD and 50 kD bands among proteins removed by this treatment were frequently detected as antigens with serum IgG/IgE antibodies from AD patients by means of IgG/IgE-immunoblotting (unpublished data). Furthermore, most of the sera from AD patients, which were positive in RAST of the original rice, proved negative in a RAST of the HRS-1 (4, 5). Thus the HRS-1 was negative regarding allergenicity for most anti-rice IgE antibodies from AD patients.

Table I. *Overall severity of AD in three age groups.*

Age (yrs)	Severity		% of severe cases
	Moderate	Severe	
0– 9 (n = 19)	8	11	42.1
10–19 (n = 13)	1	12	92.3
20– (n = 12)	0	12	100.0
Total (n = 44)	9	35	79.5

Subjects

The subjects were 44 patients with recalcitrant AD, who were suspected of having rice allergy, according to results of either elimination test, provocation test, or rice-RAST prior to the baseline evaluation. The male/female ratio of the subjects was 21:23 and their mean age was 13 years (7 months – 44 years). All cases were moderate (n = 9: 20%) or severe (n = 35: 80%). The geometrical mean of serum IgE was 2952 U/ml (min. 31, max. 50,000). Most subjects had a rice-RAST score of 2 or more (score 2: 45%, score 3: 48%).

Table I shows the frequency of each severity grade in the three age groups, and Table II the frequency of each RAST score for several allergens in the subjects.

Clinical trial

The Dermatological Pediatric and Allergy departments of 13 hospitals participated in this trial. The locations of these institutions are widely distributed in Japan: Hokkaido, Fukushima, Saitama, Tokyo, Kanagawa, Hyogo, Kochi, Fukuoka and Kagoshima prefectures. The subjects were fed with HRS-1 instead of eliminating both regular rice and wheat strictly from their daily diet, for at least 4 weeks, since AD patients with rice allergy frequently cross-react to wheat allergen, or have the non-cross-reactive wheat allergy, too. Five of the 44 subjects were considered to have been provoked by the HRS-1 diet, because in their cases a remarkable improvement had been already induced at the baseline of this study by millet and barnyard grass diet in order to eliminate the regular diet of rice and wheat. Five of the 39 subjects were provoked by the regular rice diet following this HRS-1 therapy. Elimination of egg, milk or soybean diet depended on the case, but was constant both before and throughout the study.

The extent of overall skin lesions was expressed by using the ADASI (AD Area and Severity Index), which we formulated by modifying the PASI (psoriasis area and severity index) in psoriasis as follows:

$$ADASI = 0.2(Eh + Lh + Dh + Ih)Ah + 0.4(Et + Lt + Dt + It)At + 0.2(Eu + Lu + Du + Iu)Au + 0.2(El + Ll + Dl + Il)Al$$

where

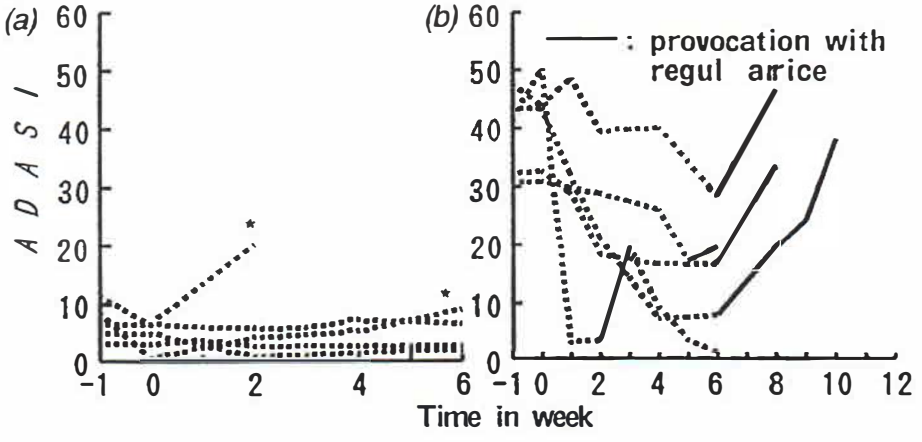

Fig. 3. Time course of ADASI during provocation with HRS-1 *(a)*, and with regular rice following the replacement treatment with HRS-1 *(b)*. The HRS-1 induced exacerbation in 2 cases (*), while regular rice caused exacerbation in all the cases tested by provocation.

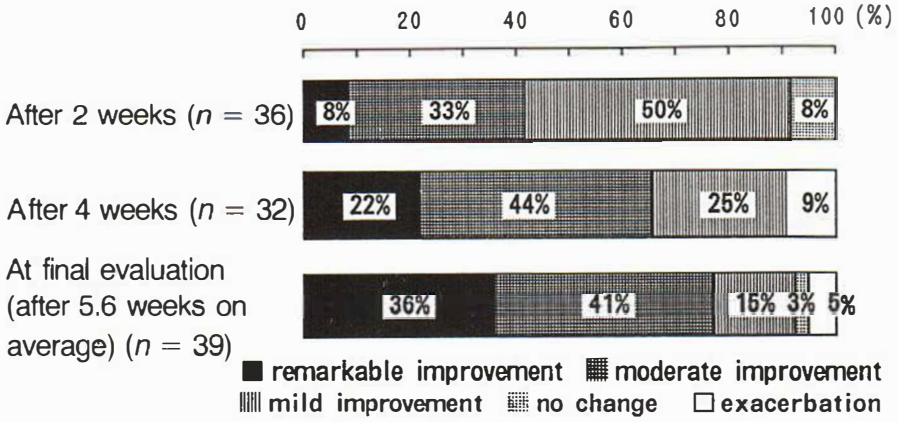

Fig. 4. Evaluation of global improvement during replacement therapy with HRS-1.

E: erythema/papule/vesicle, L: lichenification/prurigo/infiltration, D: desquamation/scale/crust, I: itching, A: area. h: head, t: trunk, u: upper extremities, l: lower extremities.

The severity of each symptom (E to I) was graded by the investigators on a 0 to 4 point scale (0: no symptom, 1: mild, 2: moderate, 3: severe, 4: very severe).

The clinical effect of HRS-1 on AD was evaluated every second week according to the ADASI, global improvement (remarkable, moderate, mild, no change, or exacerbation) and reduction of the dosage and/or potency of steroid ointment used (remarkable, moderate, mild, no change, or increase). At the end of the clinical trial, the usefulness of HRS-1 was evaluated in the 44 subjects as follows: very useful, useful, slightly useful, and questionable.

The patients and their families were asked whether the taste of HRS-1 was better, similar, or worse than regular rice, millet and barnyard grass.

Statistical analysis

ADASI was classified into five categories for non-parametric analysis, as follows:

ADASI = 0: no symptom (0), 0 < ADASI < 18: mild (1), 18 ≤ ADASI < 36: moderate (2), 36 ≤ ADASI < 54: severe (3) and 54 ≤ ADASI ≤ 72 (maximum index): very severe (4) Wilcoxon 1-sample test was used for the time-series analysis of the categorized ADASI. Dunn modulus 5% (0.0083) was adopted as a level of significance for multiple time series comparisons.

RESULTS

Time course of ADASI

A remarkable decrease in ADASI from the baseline of the replacement therapy with HRS-1 was observed in most cases as shown in Fig. 2. A transient increase in ADASI was found in 6 cases, in which other exacerbating factors such as common cold or psychologic stress were presumed to be involved. But exacerbating reflected by obvious increase in ADASI, was observed in 2 cases (5%). Non-parametric analysis of the categorized ADASI indicated a statistically significant decrease in ADASI at the 2nd and the 4th week readings and on completion of the therapy with HRS-1 (5.6 weeks, on average), as shown in Table III. In the 5 cases of provocation with HRS-1, an obvious increase in ADASI was observed in 2 cases, but none at all in 3 (see Fig. 3a). In provocation with regular rice, all of the 5 cases in which a remarkable decrease in ADASI was induced by the HRS-1 diet showed an obvious increase in ADASI just after this treatment (see Fig. 3b).

Global improvement

'Moderate' to 'remarkable' improvement was observed in 41%, 66% and 77% of the patients at the 2nd and the 4th week readings and on conclusion of the therapy, respectively, as shown in Fig. 4. Only 3 cases showed no change or exacerbation (3/39: 8%).

Reduction of steroid ointment

A 'moderate' to 'remarkable' reduction in the dosage and/or potency of steroid ointment used was observed in 31%, 43% and 59% of the patients at the 2nd and the 4th week readings and at the end of the therapy, respectively, as shown in Fig. 5. An increase was observed in only 2 cases (2/39: 5%).

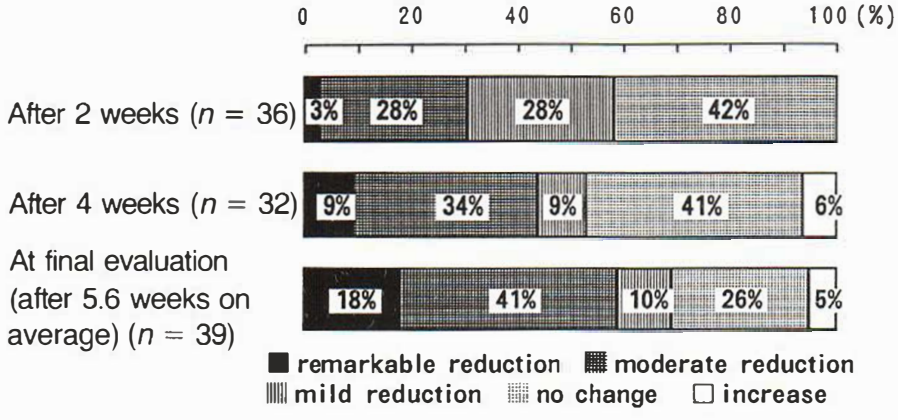

Fig. 5. Reduction effect of the replacement therapy with HRS-1 on the dosage and/or grade of steroid ointment used concomitantly.

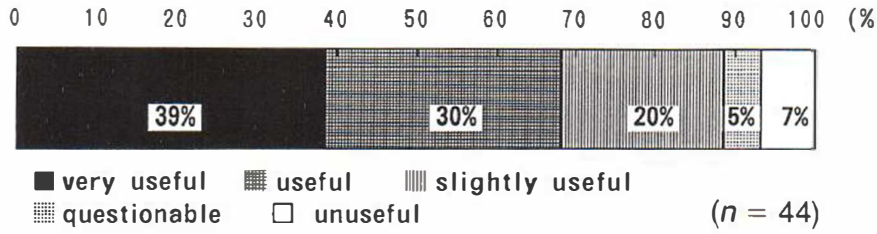

Fig. 6. Final evaluation of the clinical usefulness of HRS-1.

Usefulness of HRS-1

From the above-decribed results, the HRS-1 was finally evaluated as 'useful' to 'very useful' as the elimination diet for rice allergy in 69% of the patients, as shown in Fig. 6.

Taste of HRS-1

The frequency with which the taste of HRS-1 was better than, similar to, or worse than that of regular rice, was 11%, 72% and 17%, respectively. By contrast, the frequency with which the taste of HRS-1 was better than, similar to, or worse than that of millet and barnyard grass, was 84%, 13% and 3%, respectively.

DISCUSSION

From the results of this mass trial of HRS-1 in the recalcitrant AD patients with suspected rice allergy, it is clearly indicated that the replacement therapy with HRS-1 induced considerable improvement in most of these patients. The improvement was also confirmed by a statistically significant decrease in ADASI. Thus, the HRS-1 was found to be useful as the elimination diet for many AD patients with rice allergy. These results also suggested that the constituent proteins of 16 kD, 25 kD, 50 kD, 90 kD, etc., are major rice allergens in many patients with rice allergy. On the other hand, in this trial, we observed apparent exacerbation in 4 cases (4/44: 9%) during the HRS-1 diet. It should be considered that the constituent proteins remaining in the HRS-1 may also be major allergens in some cases with rice allergy. In fact, the sera from the patients exacerbated by the HRS-1 treatment had IgG antibodies against the constituent proteins, especially around 52 kD remaining in the HRS-1 (5). In these cases, the proteins remaining may induce allergic reactions as major allergens, resulting in the exacerbation of AD.

During this trial with HRS-1, we eliminated not only the regular rice foods but also the wheat-based foods such as bread and noodles strictly from the daily diet. Because AD patients with rice allergy frequently have wheat allergy too, and the correlation between two such allergies is revealed to be due to the partial cross-reactions between the two allergens (2, 3), as mentioned above, it is presumed that the effect of clinical improvement by HRS-1 is induced by eliminating not only rice but also wheat, and also that the constituent proteins decomposed in the HRS-1 contain the antigens cross-reacting with wheat allergens. The HRS-1 is therefore expected to be useful as the elimination diet not only for rice allergy but also for wheat allergy.

Although most of the subjects were resistant to topical steroid, 'moderate' to 'remarkable' reduction of the dosage and/or potency of steroid ointment used was observed in 59% of the patients at the end of the HRS-1 treatment. Steroid ointment is usually very effective against the eczematous lesions in AD. But it has side effects, resulting in impetigo, herpes simplex infection, rosacea-like dermatitis and skin atrophy, and its activity is apt to wane during long use. Thus a reduction of steroid use by the HRS-1 indicates that elimination of the relevant allergens is an essential strategy required for the treatment of cases of recalcitrant AD with allergy to several allergens. However, it has been very difficult to eliminate both rice and wheat from the daily diet for long periods of time without the proper dietary foods. Fortunately, the taste of HRS-1 was better than that of millet and barnyard grass, and was also similar to that of regular rice. Therefore, the HRS-1 can be expected to serve as a valuable elimination diet for AD patients with rice and wheat allergy, especially in Japan, where rice foods are consumed as the favourite staple foodstuff.

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Table II. *IgE-RAST for several allergens.*

Allergen	RAST score					RAST positive (score ≥ 1)	Geometric mean of IgE-RAST (PRU/ml)
	(4)	(3)	(2)	(1)	(0)		
Rice	0	21	20	2	1	97.7% (43/44)	3.1 (n = 39)
Wheat	0	15	19	4	6	86.3% (38/44)	2.1 (n = 38)
Soybean	0	10	20	1	12	72.1% (31/43)	1.2 (n = 38)
Egg white	6	6	11	3	14	65.0% (26/40)	2.1 (n = 35)
Milk	2	5	8	6	15	58.3% (21/36)	0.9 (n = 32)
Mite	25	4	2	0	4	68.6% (31/35)	19.4 (n = 30)

n: Number of cases tested.

Table III. *Time series analysis of categorized ADASI.*

	ADASI categorized					Total	Wilcoxon 1 sample test
	Very severe	Severe	Moderate	Mild	No symptom		
Baseline	3	12	13	8	0	36	$P_0 = 0.000008$*
After 2 weeks	0	3	12	21	0		
Baseline	3	11	12	6	0	32	$P_0 = 0.000014$*
After 4 weeks	0	2	9	21	0		
Baseline	3	13	14	9	0	39	$P_0 = 0.000004$*
After 5.6 weeks	0	1	6	32	0		

* Significant, $P_0 <$ Dunn modulus (5%) = 0.0083.

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