

Rice: Another Potential Cause of Food Allergy in Patients Sensitized to Lipid Transfer Protein

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Key Words

Food allergy · Rice · Anaphylaxis · Lipid transfer protein

Abstract

Recent studies show that the lipid transfer protein (LTP), the major Rosaceae allergen in patients not sensitized to birch pollen, is a largely cross-reacting allergen. Moreover, it is a potentially hazardous allergen due to its stability upon thermal treatment and pepsin digestion. The present study reports 3 cases of rice-induced anaphylaxis in LTP-allergic patients. In vitro inhibition studies, carried out using LTP purified from both rice and apple as well as whole peach extract, show that LTP was the relevant allergen in these patients and demonstrate the cross-reactivity between rice LTP and peach/apple LTP.

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Introduction

Lipid transfer proteins (LTP) are ubiquitous allergens in plant-derived foods belonging to the pathogenesis-related protein group 14 [1]. Cross-reactivity between LTP from botanically distinct sources may occur [2–6], particularly in patients showing high levels of IgE specific for peach LTP, the primary sensitizer to this allergen [7]. LTP

are both pepsin resistant [6] and heat stable [7–11], and are therefore potentially harmful allergens [12]; to date, systemic allergic reactions (including anaphylaxis, urticaria/angioedema and asthma) have been reported in LTP-allergic patients following the ingestion of Rosaceae [2, 5, 6, 13, 14], walnut and hazelnut [5, 6, 15–17], maize [5, 18], beer [5, 19, 20], grapes [21], mulberry [22], peanut, and mustard [5, 6]. In this study, we report 3 cases of rice-induced anaphylaxis that occurred in LTP-allergic patients; these cases show that rice should be considered as another potential cause of allergic reaction in some patients sensitized to this protein.

Patients and Methods

Case Reports

Case 1

A 50-year-old woman with a 14-year history of grass pollen hypersensitivity causing seasonal rhinoconjunctivitis and bronchial asthma had noted immediate oral itching following the ingestion of a large number of fruits and tree nuts including peach, apricot, apple, cherry, hazelnut, walnut and almond during the last 10 years (table 1). Some months before the visit she had experienced generalized urticaria and angioedema associated with dyspnea about 30 min after eating a 'risotto' dish (boiled rice with mushrooms), and about 2 months later she experienced oral allergy syndromes followed by generalized urticaria and collapse following the ingestion of a risotto with saffron. Food-specific IgE

Table 1. Main clinical features of the 3 patients

	Case 1 (female, 50 years)	Case 2 (female, 25 years)	Case 3 (male, 30 years)
Offending foods (other than rice)	Rosaceae, nuts	Rosaceae, nuts, peanuts	Rosaceae, nuts, raw tomato, onion
Offending rice dish	Risotto with mushrooms Risotto with saffron	Rice with saffron	Rice with chicory
Rice-induced symptoms	Urticaria/angioedema, dyspnea, collapse	Urticaria/angiodema, dyspnea	Urticaria
SPT commercial rice extract	Positive	Positive	Positive
Rice-specific IgE	9 kU/l	Not tested	Not tested

levels were measured (CAP-System; Pharmacia, Uppsala, Sweden): peach >100 kU/l, apple >100 kU/l, peanut 43 kU/l, hazelnut 31 kU/l, soybean 22 kU/l, orange 12 kU/l, rice 9 kU/l and wheat 8 kU/l. IgE specific for mushrooms was not measured. Skin prick tests (SPTs) with seasonal and perennial airborne allergens (Allergopharma, Reinbek, Germany) confirmed grass hypersensitivity and showed moderate skin reactivity to mugwort pollen. SPTs with commercial extracts of plant-derived foods (Abello, Lainate, Italy) showed strong skin reactivity to peach, hazelnut, walnut, sesame, sunflower, tomato and rice; in all cases the mean wheal diameter produced by these allergens exceeded that induced by an SPT with 10 mg/ml histamine. In contrast carrot, orange, potato, celery, banana, melon, wheat, maize and kiwi extracts produced weak or negative reactions. A commercial mushroom extract for skin prick testing was not available and an SPT carried out with dried mushroom (*Boletus edulis*) similar to those present in the risotto dish scored negative. An SPT with saffron powder (1 g/ml in saline) scored positive. Based on the positive SPT with commercial peach extract, the patient was diagnosed as being hypersensitive to LTP [5, 6], and was advised to avoid Rosaceae with peel (either fresh or as fruit juices, sweets or ice cream) as well as tree nuts. Based on the clinical history, SPT and in vitro assays, rice allergy was diagnosed. Due to the severity of the reported allergic reaction, the patient was not submitted to open or double-blind placebo-controlled challenges with rice.

Case 2

A 25-year-old woman with grass pollen allergy since the age of 13 started having oral allergy syndromes from Rosaceae (peach, apple, plum, cherry), walnut and hazelnut at the age of 20 (table 1). At the same age she experienced 2 episodes of generalized urticaria: one about 1 h after eating peanuts and another one after contact with a body cream containing almond milk. In 2000, SPT with commercial food extracts (Dome-Hollister/Stier, Spokane, Wash., USA) showed strong reactivity to rice, maize, peanut, walnut, tomato, almond, legumes, celery and plum. In March 2005, the patient experienced generalized urticaria and angioedema associated with dyspnea about 1 h after eating a risotto dish (boiled rice with saffron). SPT with the available commercial extracts of plant-derived foods (Abello) showed strong skin reactivity to peanut, almond, sunflower, hazelnut and peach, while legumes, carrot, orange, potato, celery, melon and banana scored negative. Unfortunately, a commercial rice extract was no longer available. An SPT with saffron (1 g/ml in saline) scored positive. Rice (and possibly saffron) allergy was diagnosed. Due to the severity of the

reported allergic reaction, the patient was not submitted to open or double-blind placebo-controlled challenges with rice.

Case 3

A 30-year-old man with a history of grass pollen allergy causing seasonal rhinoconjunctivitis since the age of 15 was recently seen (table 1). He reported oral itching following the ingestion of peach, apple, raw whole tomato (peeled, cooked tomatoes were tolerated), hazelnuts and onion since 1996. In 1998 the man started experiencing several urticaria episodes following the ingestion of peach with peel (peeled peach was well tolerated), risotto dishes (rice with chicory) and onion. He strictly avoided all these foods since then. Recently he had single episodes of laryngeal edema following the ingestion of pistachios. In 1998 he was seen by one of the authors (R.A.) at the allergy center of the Bollate hospital where SPT with commercial food allergen extracts (Dome-Hollister/Stier) showed skin reactivity to peach, peanut, legumes, tomato, walnut, rice, maize, rye and wheat exceeding that induced by 10 mg/ml histamine; SPTs with barley, oat, onion, garlic, celery, carrot, potato, orange and almond were negative or only weakly positive. The patient showed strong skin reactivity to both fresh raw and cooked apple but weak reactivity to fresh chicory. SPTs with commercial food extracts (Abello) were repeated in September 2005 and showed hypersensitivity to peach, hazelnut, walnut and peanut, whereas maize, carrot, orange, potato, almond, sunflower and banana scored negative. A commercial rice extract was no longer available.

Methods

Extraction of LTP from Rice and Apple

LTP was extracted from whole rice. A soluble extract was obtained from ground rice (250 g) as previously described [23]. The soluble proteins present in this extract were precipitated by the addition of solid ammonium sulfate up to 90% saturation. After centrifugation, the precipitated proteins were solubilized in a minimal volume of Tris-HCl 50 mM, pH 7.5, and then submitted to gel filtration chromatography on a Bio-Gel P-60 (Bio-Rad, Milan, Italy) column, which had previously been equilibrated with Tris-HCl 50 mM, pH 7.5, ammonium sulfate 0.3 M. The fractions in the elution profile of this column containing a protein with a molecular mass corresponding to that of peach recombinant LTP, as judged by SDS-PAGE, were pooled and the proteins were concentrated by ultrafiltration in an Amicon cell. Proteins, in the presence of sodium acetate 10 mM, pH 5.6, were then applied to a SP-Sepharose Fast Flow (Pharmacia) cation exchange column,

equilibrated with the same buffer, and separated by applying a linear 0–0.5 M NaCl gradient. Nearly pure LTP, as judged by SDS-PAGE, was eluted at a concentration of NaCl of about 0.35 M.

Apple LTP was purified from apple peel extract. Lyophilized extract was re-suspended in one tenth of the original volume and submitted to gel permeation chromatography on a Superdex 75 column (Pharmacia) equilibrated in PBS/0.05% NaN₃ (bed volume: 150 ml). Fractions were analyzed by SDS-PAGE. Fractions exclusively containing the 9-kDa LTP band were pooled, dialyzed against 5 mM ammonium bicarbonate and lyophilized [5]. Protein concentrations, measured according to Bradford [24] by the Bio-Rad method (Bio-Rad), were 2,130 and 1,000 µg/ml for rice and apple LTP, respectively.

Peach Peel Extract

Peach peel was homogenized and extracted as previously described [25]. The protein concentration of the extract, measured according to Bradford [24], was 750 µg/ml.

Whole Rice Extract

Both raw and cooked rice were homogenized and soluble proteins extracted in 0.1 M PBS, pH 7.4, by shaking for 4 h at 4°C. The suspensions were centrifuged at 30,000 g for 30 min at 4°C and the corresponding supernatants filtered through a 0.45-µm filter. Protein concentrations, measured according to Bradford [24], were 668 µg/ml for raw rice and 118 µg/ml for cooked rice.

Measurement of Specific IgE by ELISA and ELISA Inhibition Experiments

IgE specific for whole rice extract (both raw and cooked), purified rice LTP and apple LTP were measured in sera from patients 1 and 2, in a serum pool of the 3 patients, and in a control serum pool (a pool of sera from 5 nonatopic individuals) by ELISA. Two micrograms of raw rice extract, 2 µg of cooked rice extract, 0.2 µg of rice LTP or 0.1 µg/100 µl of apple LTP, all diluted in 100 µl of coating buffer (15 mM Na₂CO₃ and 35 mM NaHCO₃, pH 9.6) per well, were used for coating 96-microtiter plates (Maxisorp; Nunc, Roskilde, Denmark). After washings with 0.1 M PBS, pH 7.4, and 0.05% Tween 20 (Sigma, Milan, Italy), the wells were saturated with 2% BSA in PBS (dilution buffer) for 2 h at room temperature. After further washing, 100 µl of the normal serum pool or patients' serum (both 1:3 in dilution buffer) were added to the wells and incubated for 2 h at room temperature. The wells were washed again and bound specific IgE was detected by peroxidase-conjugated anti-human IgE from goat (dilution 1:1,500; Biospecific, Emeryville, Calif., USA); the colorimetric reaction was induced using tetramethyl-benzidine/H₂O₂ as substrate. The enzyme reaction was stopped after 20 min adding 1 M HCl. Absorbance values were read at 450 nm by a spectrophotometer. In inhibition studies, patients' sera (1:3 in dilution buffer) were absorbed for 2 h at room temperature with different amounts (1.7, 0.34 and 0.068 µg) of peach peel extract, 0.2 µg of rice LTP or 20 µg of house dust mite extract (4,800 µg/ml) before the test. IgE levels were expressed as optical density units (OD). In a further inhibition study, the IgE reactivity of patient 1 to whole raw rice extract was inhibited by preabsorption with either 0.1 µg of rice LTP or 20 µg of whole raw rice extract.

Finally, the IgE reactivity of the serum of patient 1 against rice LTP in the presence of different concentrations of commercially available rice starch was assessed. In these experiments, rice

starch (0.5 or 2.5%) was added to the buffer containing 1 mg/ml of rice LTP about 30 min before the microtiter wells were coated. ELISA was subsequently carried out as previously described.

Based on the mean + 2 SD of IgE levels found in normal controls, values <300 OD were considered negative.

Immunoblot and Immunoblot Inhibition Experiments

Sera from patients 1 and 2 were tested against both cooked and raw rice extracts by immunoblot under reducing conditions. Both extracts were mixed with LDS sample buffer (Nupage Bis-Tris Novex; Prodotti Gianni, Milan, Italy) and 5% β-mercaptoethanol. The samples were then denaturated by heating at 100°C for 5 min. Electrophoresis of cooked and raw rice extract (25 µg/lane) was carried out in a 10% polyacrylamide precast gel (Nupage Bis-Tris Novex; Prodotti Gianni, Milan, Italy) at 180 mA for 1 h. The resolved proteins were transferred onto a nitrocellulose membrane for 1 h according to Towbin et al. [26]. The membrane was saturated with 0.1 mol/l Tris-buffered saline containing 5% fat-free milk powder and incubated for 16 h at 4°C with sera. After 3 washings, bound specific IgE were detected by peroxidase-conjugated anti-human IgE antibodies from goat (1:1,000 in saturation buffer) and using an ECL Western blotting kit (Amersham, Milan, Italy) as substrate. The IgE reactivity to rice LTP of the serum pool from the 3 patients was also investigated by immunoblot analysis and immunoblot inhibition studies. In these studies, 7 µg/lane of rice LTP was employed; in inhibition studies, the IgE reactivity was inhibited by preabsorption of the serum pool with either 285 or 57 µg of peach peel extract.

Results

ELISA and ELISA Inhibition

Patient 1 showed strong IgE reactivity to both rice LTP and apple LTP (1,958 and 1,857 OD, respectively). In inhibition experiments, the preabsorption of the serum with increasing amounts of peach peel extract caused a gradual reduction of IgE reactivity to rice LTP that was eventually abolished at 1.7 µg of peach extract (fig. 1). The preabsorption with house dust mite extract did not exert any effect on serum IgE reactivity to rice LTP, that was conversely totally abolished by preabsorption with 0.2 µg of rice LTP. Further, the patients' IgE reactivity to whole raw rice extract was totally abolished by preabsorption of the serum with rice LTP (table 2).

Patient 2 showed a strong IgE reactivity to apple LTP (1,688 OD) and a moderate IgE reactivity to rice LTP (559 OD) that was inhibited only by preabsorption of the serum with rice LTP or the highest concentration of peach peel extract (1.7 µg; fig. 2).

The sera from both patients did not show any reactivity to the whole cooked rice extract, and only patient 1 reacted to the whole raw rice extract but much less intensely than to rice LTP (table 2).

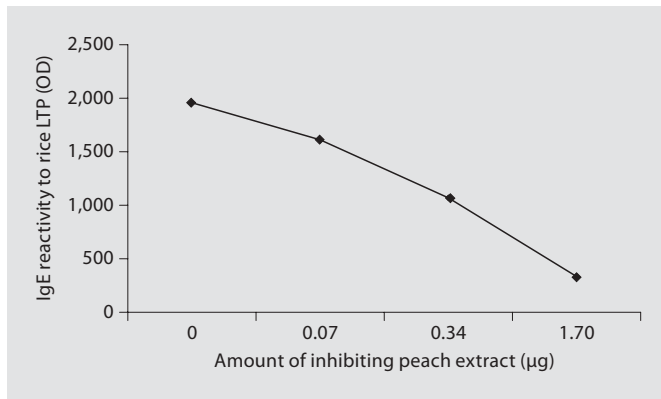


Fig. 1. ELISA inhibition studies with serum from patient 1. The IgE reactivity to rice LTP is progressively abolished by preabsorption with increasing doses of peach peel extract.

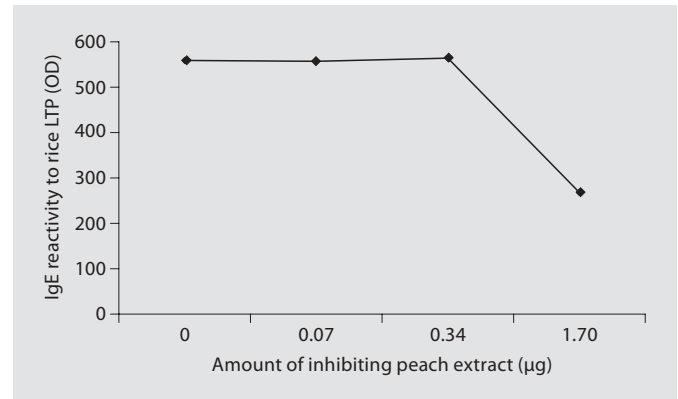


Fig. 2. ELISA inhibition studies with serum from patient 2. The IgE reactivity to rice LTP is abolished by preabsorption of the serum with the highest dose of peach peel extract.

Table 2. ELISA and ELISA inhibition

	Case 1	Case 2	Normal
IgE to 2 µg of raw rice extract	831	242	225
IgE to 2 µg of cooked rice extract	224	198	205
IgE to 0.1 µg of apple LTP	1,857	1,688	67
IgE to 0.2 µg of rice LTP	1,958	559	48
IgE to rice LTP after absorption of serum with 1.7 µg of peach extract	334 (82)	268 (52)	
IgE to rice LTP after absorption of serum with 0.34 µg of peach extract	1,069 (45)	564 (0)	
IgE to rice LTP after absorption of serum with 0.068 µg of peach extract	1,611 (18)	557 (0)	
IgE to rice LTP after absorption of serum with 0.2 µg of rice LTP	55 (97)	293 (47)	
IgE to rice LTP after absorption of serum with 20 µg of house dust mite extract	1,867 (5)	689 (0)	
IgE to whole raw rice extract after absorption of serum with 20 µg of whole raw rice	134 (84)		
IgE to whole raw rice extract after absorption of serum with 0.1 µg of rice LTP	156 (81)		

Specific IgE are measured in OD. Values <300 OD are considered negative. Figures in parentheses represent the percentage of inhibition.

The 3 patients' serum pool showed an intense reactivity to rice LTP (2,106 OD). The normal serum did not show any reactivity to LTP (48 OD).

The presence of rice starch clearly hampered the binding of specific IgE of patient 1 to rice LTP. IgE reactivity was 2,094 OD, 1,112 OD and 532 OD in the absence of rice starch or in the presence of 0.5 and 2.5% of rice starch, respectively.

Immunoblot and Immunoblot Inhibition

SDS-PAGE of both raw and cooked rice extracts is shown in figure 3. Interestingly, several protein bands

(including the one at about 10 kDa, corresponding to the molecular weight of LTP) present in the raw rice extract were no longer visible in the cooked rice extract. Sera from both patients 1 and 2 scored negative when tested by immunoblot against raw and cooked rice under reducing conditions (data not shown). In contrast, the serum pool of the 3 patients showed a clear-cut IgE reactivity against rice LTP (fig. 4); the pool of normal sera did not show any reactivity to the protein. Preabsorption of the patients' serum pool with 57 µg of peach peel extract was sufficient to abolish IgE reactivity to rice LTP (fig. 4).

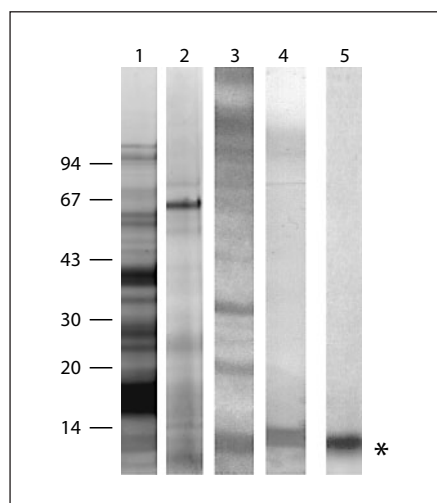


Fig. 3. SDS-PAGE. Lane 1: raw rice extract. Lane 2: cooked rice extract. Lane 3: peach peel extract. Lane 4: rice LTP. Lane 5: apple LTP. Several bands are lost in the whole rice extract after thermal processing. The asterisk shows the molecular weight of LTP.

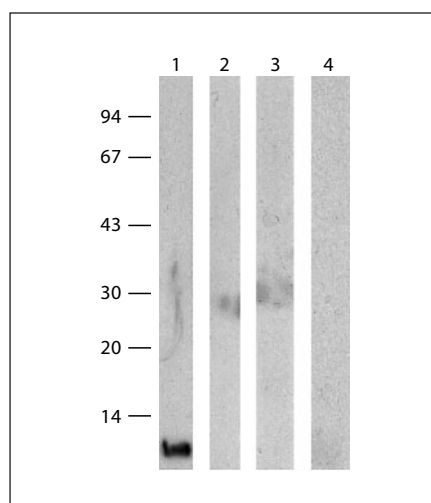


Fig. 4. Immunoblot and immunoblot inhibition experiments with rice LTP. Lane 1: uninhibited patients' serum pool. Lanes 2 and 3: patients' serum pool preabsorbed with 285 and 57 µg of peach extract, respectively. Lane 4: control serum pool.

Discussion

This study shows for the first time that some patients allergic to LTP may experience severe allergic reactions eating rice. Both ELISA and immunoblot inhibition studies confirmed the cross-reactivity between peach and rice LTP and showed that in these patients IgE reactivity to rice was totally associated with sensitization to LTP. Based on previous studies of the relevance of LTP-specific IgE levels for the development of clinical symptoms [7], it is conceivable that cross-reactivity to rice may occur in some patients showing high levels of IgE to peach LTP; in effect, one of our patients showed >100 kU/l of IgE against both peach and apple on CAP-System measuring. All 3 patients scored strongly positive on SPT with commercial rice extract, suggesting that the *in vivo* test is useful as a means of detecting LTP-allergic patients who are prone to react to this food as well. In spite of the positive SPT, the sera from 2/2 of our patients did not show any reactivity to whole cooked rice neither on ELISA nor on immunoblot analysis. The reasons for this are unclear. The possibility that rice LTP is heat labile seems unlikely as all studies of this protein carried out so far have clearly shown that LTP is extremely heat resistant [8–11]. A dilution of the allergen during cooking procedures beyond the detection limits of *in vitro* assays cannot be excluded. Alternatively, the incorporation of LTP into a macromolecular matrix (e.g. starch) during cooking procedures

that may hamper the interaction between the allergen and specific IgE may be another possible explanation. In effect, clinical allergy to cereals (maize, barley, wheat, spelt and rice) in patients sensitized to LTP seems rather uncommon and has been reported only in selected cases. Further, our *in vitro* experiments showed that the presence of rice starch greatly reduced the IgE reactivity to rice LTP. *In vivo* such a matrix might be digested in the gastrointestinal tract, making LTP recognizable again by specific antibodies. Finally, the fact that both sera scored negative on immunoblot with whole raw rice extracts also suggests that the amount of LTP in rice extract is probably rather small, possibly below the detection limit of this technique.

In conclusion, the present study suggests that rice should be included in the list of plant-derived foods potentially able to induce allergic reactions in patients sensitized to LTP.

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