

TABLE I. The mean (range) of outputs from characterized De Vilbiss 646 nebulizers using the KoKo Digidoser and the Rosenthal Dosimeter

Nebulizer no.	Manufacturer-labeled output (mL/min)	Digidoser (mL/min)	Rosenthal Dosimeter rebreathe method* (mL/min)	Rosenthal Dosimeter standard method† (mL/min)
1250	0.883	0.327 (0.274-0.351)		
1251	0.886	0.473 (0.302-0.735)		
1538	0.965	0.579 (0.515-0.699)		
1541	0.830	0.178 (0.139-0.203)		
3634	0.901	0.256 (0.208-0.307)	0.912 (0.860-0.980)	1.50 (1.42-1.56)
3856	0.841	0.233 (0.186-0.275)	0.800 (0.520-0.960)	1.42 (1.36-1.54)
3900	1.028	0.491 (0.346-0.626)		
Mean (SD)	0.897 (0.06)	0.362 (0.15)‡	0.856 (0.07)	1.46 (0.05)§

*With the rebreathe method, the operator exhales into the nebulizer.

†With the standard method, the operator exhales into the room air.

‡Significantly lower than the manufacturer-labeled output ($P < .0001$).

§Significantly higher than the manufacturer-labeled output ($P < .0001$).

reduced by about one-half concentration⁵; that is, increasing the flow, pressure, or both so that the nebulizer delivers 0.9 mL/min increases the amount of methacholine delivered and therefore reduces the apparent PC₂₀. As a consequence, the standard method, which also produces less variability than the rebreathe method, has been recommended for calibrating nebulizers.^{5,6} However, only the rebreathe method can be used with the Digidoser because the system will not trigger the next activation if the subject does not exhale into the nebulizer.

We conclude that when using the KoKo Digidoser, methacholine PC₂₀ values of 8 mg/mL or greater should be interpreted with caution.

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Lipid transfer protein is involved in rhinoconjunctivitis and asthma produced by rice inhalation

To the Editor:

In spite of the fact that rice is one of the most consumed cereals worldwide, few cases of true rice allergy have been reported. Two different routes of exposure leading to immediate hypersensitivity reactions have been documented: ingestion of the cooked cereal¹ and inhalation of vapors during its boiling.² Besides a 33-kd glyoxalase I, the major rice seed allergens so far characterized belong to the 14-kd to 16-kd α -amylase inhibitor family of cereals.^{1,2} Wheat and rye members of this protein family are also involved in baker's asthma, caused by the occupational inhalation of these flours.³

Three patients with respiratory allergy to raw rice by inhalation were included in this study (Table I). All of them showed an acute allergic reaction (rhinoconjunctivitis or asthma) while they threw rice in different weddings. Two of them had a pollinic background, and all 3 patients were allergic to peach but tolerated rice by ingestion. Positive specific IgE determination (CAP system FEIA; Pharmacia Diagnostics, Uppsala, Sweden) and skin prick test (SPT) responses using commercial extracts (ALK-Abelló, Madrid, Spain) to rice and peach were obtained in all patients. SPTs, IgE immunodetection, and CAP inhibition assays were also performed using peach Pru p 3, which was isolated and quantified as previously reported,⁴ and a rice seed extract made in house. Such extract was obtained from defatted rice flour extracted with PBS buffer, and after centrifugation, the supernatant was dialyzed against H₂O and freeze-dried. Immunodetection with

TABLE I. Demographic and clinical data of the rice allergic patients

Patient	Age/sex	Symptoms to rice ¹	Allergy to pollens ^{1,2}	Allergy to plant foods (symptoms) ³	Specific IgE (kU/L)	
					Rice	Peach
1	30/M	R and A	R and A (Pa)	P, tN (OAS, U)	1.12	10.00
2	41/F	R	No	P, tN, L (OAS, An)	23.80	79.80
3	26/M	R	R (Pj)	P, tN, L, M (U)	3.07	30.90

R, rhinoconjunctivitis; A, asthma; Pa, *Platanus acerifolia*; Pj, *Parietaria judaica*; P, peach; tN, tree nuts; L, lettuce; M, maize; OAS, oral allergy syndrome; U, urticaria; An, angioedema.

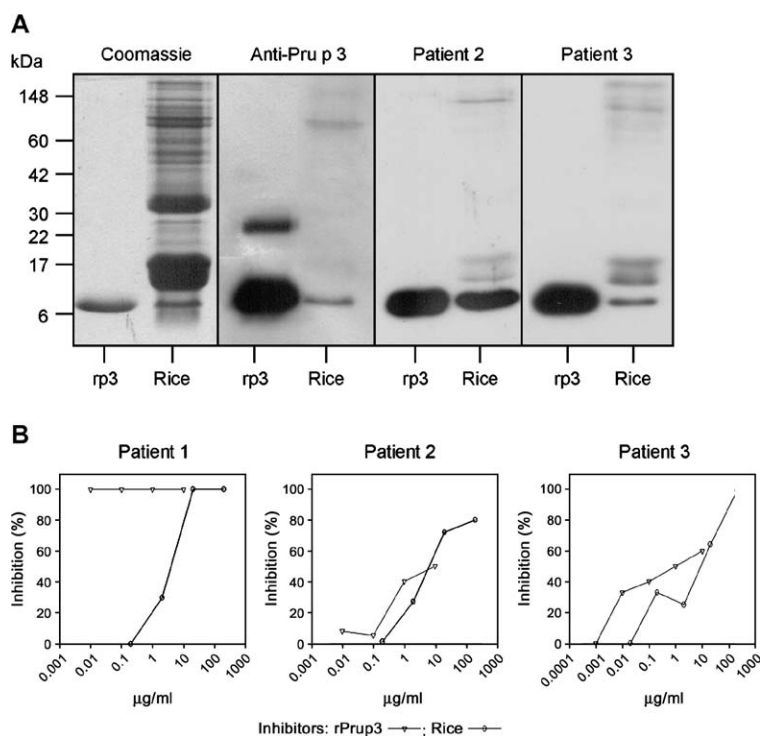


FIG 1. **A**, Protein staining (Coomassie) and immunodetection of purified recombinant Pru p 3 (rp3) and crude rice extract (Rice) with an anti-Pru p 3 antiserum and IgE from sera from patients 2 and 3 (Table I). **B**, CAP-inhibition assays of rice by a crude rice extract and purified peach recombinant Pru p 3. Individual sera from patients with rice allergy (Table I) were used.

rabbit anti-Pru p 3 antibodies⁴ and individual sera (1:4 dilution) was performed as previously described.⁵

Taking into account that peach is the major plant food causing allergic reactions in the adult Spanish population, with a lipid transfer protein (LTP; Pru p 3) as its major allergen,⁶ as well as the allergenic history of the 3 patients (Table I), a potential role of LTPs in raw rice respiratory allergy was evoked. This presumption was further sustained by the presence of a LTP in rice seeds⁷ (53% of amino acid sequence identity to Pru p 3), which was recognized by rabbit anti-Pru p 3 antibodies in rice seed extracts (Fig 1, A). Recombinant Pru p 3 as well as the rice seed LTP bound IgE (Fig 1, A) from sera of patients 2 and 3 (Table I). Additional IgE-binding bands of approximately 10 to 16 kd and 60 to 90 kd were detected in both sera. Unfortunately, serum from patient 1 was not available for immunodetection. Positive SPT responses to natural Pru p 3 (20 µg/mL) were also obtained in all 3

patients. Furthermore, CAP inhibition assays were performed with individual sera from the 3 patients (Fig 1, B), using commercial rice extract as solid phase (Pharmacia Diagnostics) and a rice seed extract made in house (250 µg/mL) or purified recombinant Pru p 3 (10 µg/mL) as inhibitors. Assays were performed as indicated in the CAP system manual, with duplicate samples of 20 µL of the undiluted serum of each patient premixed with 30 µL of 5 progressive 1:10 dilution of each inhibitor solution in PBS, pH 7.4, containing 0.03% (wt/vol) BSA, and BSA and a *Dermatophagoides pteronyssinus* commercial extract (ALK-Abelló) as negative controls. Inhibition values over 50% were displayed by recombinant Pru p 3 in all 3 sera, reaching a 100% inhibition in the case of patient 1 (Fig 1, B).

Lipid transfer proteins are 9-kd proteins that form a panallergen family widely distributed in fruits, vegetables, and pollens.⁶ Their high resistance to heat treatment and

proteolytic digestion together with their association with systemic and severe symptoms have led researchers to propose LTPs as a model of true plant food allergens.⁶ Furthermore, LTPs are the major allergens of *Rosaceae* fruits and other plant-derived foods in the Mediterranean, but not in the Central and Northern European population.⁶ Regarding cereal seeds, members of this panallergen family have been identified in most of them, but their role on cereal allergy, either by ingestion or inhalation, is not well established yet. Only in the cases of maize⁸ (*Zea m 14*) and beer^{5,9} (processed barley LTP) has the corresponding allergenic LTP been isolated and assayed. Interestingly, in a case report on beer allergy involving LTP as major allergen,⁹ ELISA inhibition values around 90% were reached using rice extract as inhibitor and peach Pru p 3 in the solid phase. Finally, a striking question arises from the tolerance to ingest boiled rice of the 3 patients studied here, as was noted in other cases of rice allergy by inhalation.^{2,10} LTPs are resistant to heat treatment, and therefore, active allergenic forms would be expected after rice boiling. A potential modification of some cereal LTPs by food processing, together with a greater allergenic potency by inhalation than by ingestion, could be claimed and remains to be investigated.

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