

LETTER TO THE EDITOR

Anaphylaxis to lipid transfer protein from butterbur scape

Dear Editor,

Butterbur (*Petasites japonicus*) is a dioecious perennial plant of the Asteraceae family. Butterbur scape (Figure 1a,b) is a butterbur bud and cooked in dishes such as *tempura* (deep-fried vegetables), which is unique in that it is eaten with its pollen.

A 33-year-old woman had an acute lip and oral mucosal swelling following ingestion of butterbur scape *tempura*, then developed urticaria and dyspnea. The patient has a medical history of atopic dermatitis, urticaria, and pollinosis to Asteraceae plants (chrysanthemum, mugwort, and ragweed) and has allergies to apple, melon, peach, and soy milk. Skin examination showed scattered wheals over the body. The symptoms disappeared with 300 mg i.v. hydrocortisone sodium

phosphate. ImmunoCAP (Thermo Fisher Scientific) specific immunoglobulin (Ig)E testing showed class 4 for mugwort pollen; class 3 for chrysanthemum pollen, dandelion pollen, ragweed pollen, alder pollen, and birch pollen; and class 2 for apple, peach, and wheat. Skin prick test (SPT; see Supporting Information) was positive for the non-heated male plant of butterbur scape (Figure 1c). These led to a diagnosis of anaphylaxis by butterbur scape ingestion. No symptoms have recurred by avoiding subsequent ingestion.

Western blotting using the patient's serum was performed (see Supporting Information). Serum from a healthy volunteer was used for the negative control, and sera from two individuals who had been diagnosed with butterbur scape allergy were used as positive controls

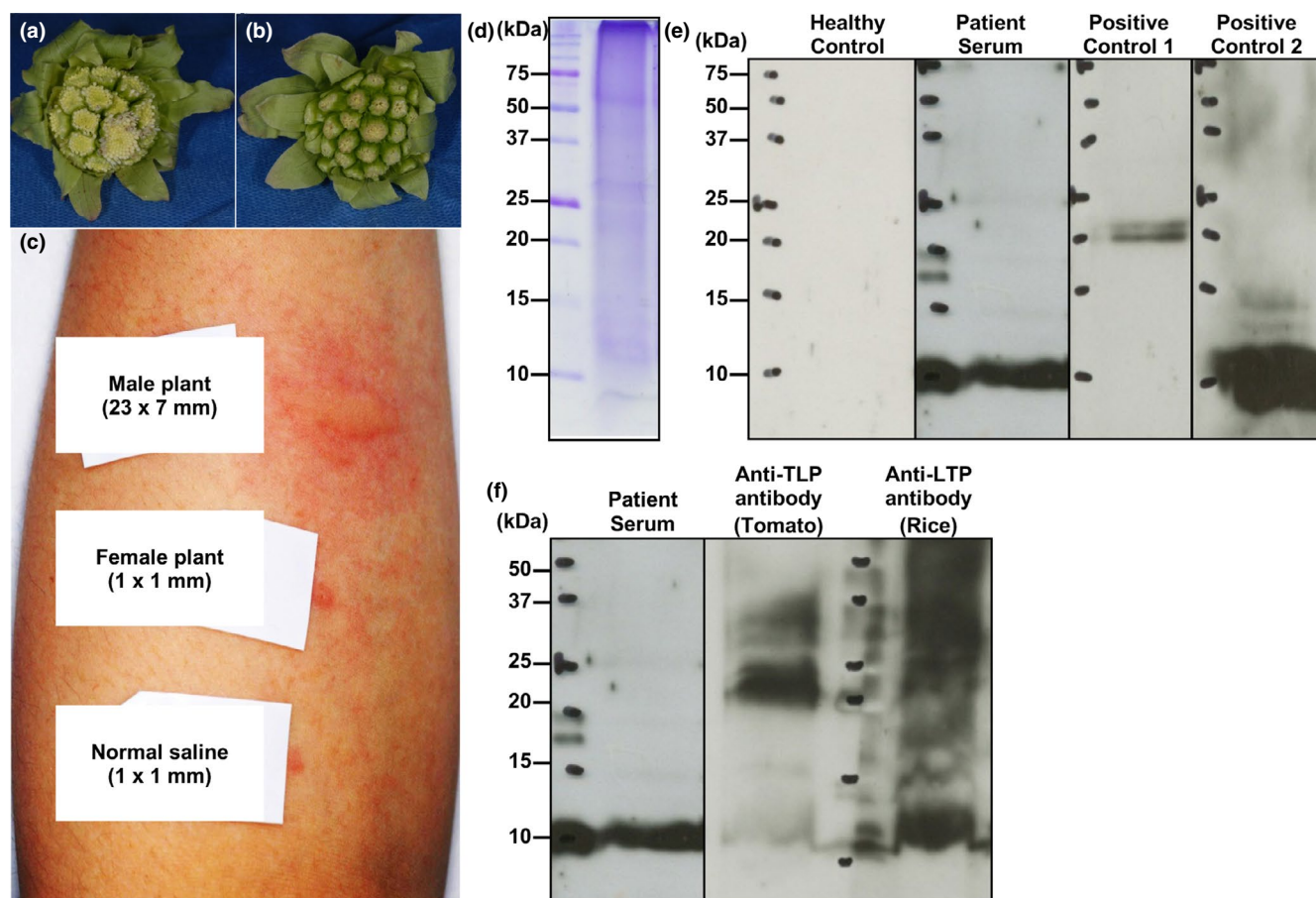


FIGURE 1 (a) Male plant of butterbur scape. (b) Female plant of butterbur scape. (c) Skin prick test was positive for the male plant of butterbur scape. Skin prick test was considered positive when the mean wheal diameter was more than 3 mm. (d) Total protein staining of the whole bodies of the male plants of butterbur scape with Coomassie brilliant blue. (e) Western blotting of butterbur scape protein fractions in the sera of healthy control, the patient, and positive controls. The IgE in the patient's serum reacted to 10-kDa protein. (f) Western blotting of butterbur scape protein fractions in the sera of the patient, anti-tomato TLP antibody, and anti-rice LTP antibody

(see Supporting Information).¹ Anti-rice lipid transfer protein (LTP) antibody² and anti-tomato thaumatin-like protein (TLP) antibody (see Supporting Information) were used to detect LTP and TLP in butterbur scape, respectively. Informed consent was obtained from the patient, and our institutional review board approved the study. CBB Stain One (Nacalai Tesque) was used for total protein staining (Figure 1d). Western blotting showed that the IgE antibody of the patient reacted to 10-kDa protein and IgE antibodies of positive controls reacted to 10- and 20-kDa proteins (Figure 1e). Moreover, anti-rice LTP antibody reacted to 10-kDa band, which was the same as the response of the patient's serum to butterbur scape (Figure 1f).

We identified LTP as an allergen in butterbur scape. Since LTP is a potent pan-allergen resistant to digestion and heat denaturation, butterbur scape allergy caused severe systemic symptoms despite being heated.^{1,3,4} Consistently, the previous report showed that SPT was positive for the heated male plant of butterbur scape.¹ The sensitization pathway of butterbur scape is currently unknown. We cannot exclude the possibility of gastrointestinal sensitization because LTP is stable for digestion,³ but we believe that sensitization occurred through the nasal mucosa as pollen-food allergy syndrome. Like this case, most previous cases had pollinosis and high serum IgE levels against Asteraceae plant pollen.⁴ Moreover, the patient's allergy history to peach, which contains peach LTP (Pru p 3),⁵ supports our hypothesis. We expect that LTP is contained in the pollen because SPT was positive only for the male plant, while negative for the female plant.^{1,4} Selective ingestion of the female plant would prevent anaphylaxis.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

None declared.

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REFERENCES

1. Kagatani S, Tsunoda T, Moriyama T. [Two cases of oral allergy syndrome to "Fukinoto"]. *[jpn J Dermatol]*. 2006;116:331-4 (in Japanese).
2. Lang GH, Kagiya Y, Ohnishi-Kameyama M, Kawamoto S, Moriyama T, Kitta K. Immunoblotting analysis of nsLTP1 in cereal grains with antiserum raised against recombinant rice nsLTP1. *Food Sci Technol Res*. 2011;17:499-504.
3. Pastorello EA, Pompei C, Pravettoni V, Farioli L, Calamari AM, Scibilia J, et al. Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100°C, as demonstrated in anaphylactic patients and patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol*. 2003;112:775-83.
4. Yaguchi Y, Tsunoda T, Moriyama T, Suzuki T. [Clinical and biochemical evaluation of eleven patients with Japanese butterbur allergy]. *[pract. Dermatol]*. 2017;39:1040-3 (in Japanese).
5. Fernández-Rivas M, González-Mancebo E, Rodríguez-Pérez R, Benito C, Sánchez-Monge R, Salcedo G, et al. Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *J Allergy Clin Immunol*. 2003;112:789-95.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

SUPPLEMENTARY MATERIALS AND METHODS

Skin prick test (SPT)

SPT was carried out on the lower aspect of the patients' forearm. Normal saline was used as a negative control. The results were recorded after 15 minutes. The mean wheal diameter was calculated according to the formula $(D + d)/2$, where D represents the largest longitudinal diameter and d its midpoint orthogonal diameter. The mean wheal diameter of more than 3 mm was considered positive.

Origin of positive control serum

The positive control sera were those that we have previously reported as butterbur scape allergy.¹ Positive control 1 was a 50-year-old woman with a medical history of pollinosis to mugwort, ragweed, and cedar. The patient was diagnosed with anaphylaxis by butterbur scape by positive SPT for the male plant of heated butterbur scape. Positive control 2 was a 36-year-old woman with a medical history of bronchial asthma and had positive results of ImmunoCAP specific immunoglobulin E testing to mugwort pollen and cedar pollen. The patient was diagnosed with oral allergy syndrome by butterbur scape by positive SPT for the male plant of non-heated butterbur scape.

Preparation of anti-tomato thaumatin-like protein (TLP) antibody

The rabbit polyclonal antibody against recombinant NP24 (tomato TLP, GenBank: M21346.1) was obtained by immunizing rabbit at Scrum Inc. (Tokyo, Japan) with recombinant NP24 prepared in our lab. Recombinant NP24 was expressed in SHuffle T7 *E. coli* strain (New England Biolabs, Ipswich, MA, USA) using expression vectors (pET-30 Xa/Lic plasmid; MilliporeSigma, Burlington, MA, USA) cloned cDNA of NP24 via PCR from a tomato leaf cDNA library. The expressed recombinant NP24 protein, fused with the 6 × His tags, was extracted through ultrasonic disruption of the cells. The protein was purified by metal-chelate affinity chromatography (GE Healthcare Life Sciences, Piscataway, NJ, USA) using a column that binds the 6 × His tags.

Western blotting

The whole bodies of the male plants of butterbur scape were homogenized with 70 ml of buffer (30 mM Tris HCl, 1mM EDTA) and filtered through gauze. The extracted butterbur scape protein separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was transferred onto a Hybond-P polyvinylidene difluoride (PVDF) transfer membrane (MilliporeSigma). The transferred membrane was blotted with serum diluted 1:10 in phosphate-buffered saline (PBS), anti-rice LTP antibody,² or anti-tomato TLP

antibody overnight at 4°C, and with the appropriate secondary antibodies for one hour at room temperature.

SUPPLEMENTARY REFERENCES

1. Kagatani S, Tsunoda T, Moriyama T. [Two Cases of Oral Allergy Syndrome to "Fukinoto"]. [Jpn J Dermatol] 2006; 116:331-4 (in Japanese).
2. Gang-Hua L, Kagiya Y, Ohnishi-Kameyama M, Kawamoto S, Moriyama T, Kitta K. Immunoblotting Analysis of nsLTP1 in Cereal Grains with Antiserum Raised against Recombinant Rice nsLTP1. Food Sci Technol Res 2011; 17:499-504.