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Original

Allergy to fig (*Ficus carica*)

Background: The fig, the fruit of the fig tree, belongs to the *Moraceae* family and is a frequently consumed foodstuff in our area. Only a few cases of hypersensitivity reactions after the ingestion of figs have been reported. **Material and methods:** Eight patients with allergic symptoms after fig ingestion were selected for this study. Skin tests were performed with fig (a commercial extract and prick-by-prick with the fresh fruit) and also with related allergens (*Ficus benjamina*, mulberry pollen, latex, kiwi, papain and bromelin); the fig-specific IgE was quantified (enzyme-linked immunoassay), and SDS-PAGE immunoblotting with fig extract was performed. An oral challenge test was carried out in five cases. **Results:** The prick-by-prick test with fresh fig was positive in all eight patients, and none of them had a positive reaction to the commercial extract. The specific IgE levels were positive in six patients. The immunoblot revealed the presence of IgE-binding proteins with molecular weights ranging between 14 and 90 kDa. The skin tests with related allergens were positive with *Ficus benjamina* in all eight patients, with kiwi in 3, and with latex and papain in one patient each. The challenge test was positive in the five cases in which it was performed. **Conclusions:** Allergy to fig should be considered in countries where this fruit is frequently consumed. The prick-by-prick test with the fresh fruit is the most sensitive method for detection. In patients with fig allergy, sensitization to other related allergens must be ruled out.

Key words: *Ficus carica*. Fig. Food allergy.

Alergia al higo (*Ficus carica*)

Introducción: El higo, fruto de la higuera (*Ficus carica*), perteneciente a la familia de las *Moraceae*, es frecuentemente consumido en nuestro medio; sin embargo, se han descrito pocos casos de hipersensibilidad tras su ingesta. **Material y métodos:** Se seleccionaron ocho pacientes con sintomatología alérgica tras la ingesta de higos. Se efectuaron pruebas cutáneas con higo (extracto comercial y prick-by-prick con la fruta fresca) y con alérgenos relacionados (*Ficus benjamina*, polen de morera, látex, kiwi, papaína y bromelina); se cuantificó la IgE específica a higo (enzimoinmunoensayo) y se llevó a cabo SDS-PAGE immunoblotting con extracto de higo. Se realizó provocación oral en cinco casos. **Resultados:** El prick-by-prick a higo fue positivo en todos los pacientes, mientras que en ninguno de ellos fue positivo el extracto comercial. La IgE específica fue positiva en seis pacientes. El immunoblotting identificó bandas proteicas fijadoras de IgE de masa molecular entre 14 y 90 kDa. Las pruebas cutáneas

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con alérgenos relacionados fueron positivas con *Ficus benjamina* en los ocho pacientes, con kiwi en tres, con látex en uno y con papaína en uno. El test de provocación fue positivo en los cinco casos en que se realizó. **Conclusiones:** La alergia al higo debe ser tenida en cuenta en aquellos países en los que esta fruta se consume habitualmente, siendo el *prick-by-prick* con el alimento fresco el método más sensible para detectarla. En estos casos debe estudiarse la existencia de sensibilización a otros alérgenos relacionados.

Palabras clave: Alergia alimentaria. *Ficus carica*. Higo.

INTRODUCTION

The fruit of *Ficus carica*, the fig tree -a member of the *Moraceae* family- is a frequently consumed foodstuff throughout the Mediterranean basin and in the Middle East. Despite this, only recently has the fig been implicated as a cause of food allergy. The first description was that of Dechamp et al.¹ in 1995, and sporadic cases of allergic reactions have been reported since then²⁻³. Sensitization to fig (positive skin tests or specific IgE) have also been reported in patients who were allergic to *Ficus benjamina* (Fb) and in whom studies of cross reactivity to related allergens were being performed (such as the fig itself, kiwi, mulberry pollen, latex, papain and bromelain)⁴⁻⁷.

We present here eight patients who had developed allergic reactions after the ingestion of fig; the specific IgE-binding proteins in the fig are also identified.

PATIENTS AND METHODS

Patients

We have studied six patients who had evidenced symptoms compatible with an allergic reaction after the ingestion of figs, and two further patients (Nos. 4 and 5 in Table I) with a diagnosis of allergy to Fb and who had had positive prick-by-prick and oral challenge tests to that fruit. Table I shows the clinical features of the eight patients.

Methods

Skin prick tests were performed with fig, *Ficus benjamina* sap, kiwi, papain, bromelain, latex and mulberry pollen. Commercially-acquired extracts were used with the exception of Fb, in which case an extract prepared from the leaves of the plant was used (Bial-Aristegui, Bilbao, Spain). Prick-by-prick tests were also performed with fresh fig flesh. Skin tests were also performed with pneumoallergens common in our environment (house dust mites, cat and dog danders, *Alternaria*, *Penicillium*, *Aspergillus* and *Cladosporium*, and *Parietaria*, *Corylus*, *Cupressus*, *Platanus*, *Artemisia*, *Olea*, *Mercurialis*, *Phragmites*, *Cynodon*, *Chenopodium* and *Plantago* pollens). Histamine hydrochloride (10 mg/ml) and normal saline were used as positive and negative controls, respectively, and the tests were considered positive when the wheals were greater than 3 mm in diameter with negative normal saline tests. Prick-by-prick tests with fresh fig flesh were performed on ten atopic control patients who normally tolerated the ingestion of figs.

Open controlled oral challenge tests were proposed, with previous full explanation and informed consent in writing, to all patients with the exception of patient No. 3 (who was pregnant) and patient No. 8 (for ethical reasons).

Because of the gelatinous nature of the fig flesh and of the difficulties this might cause in the preparation of a protein-enriched extract, the skin and the flesh of the fig were used separately in the preparation. This particular situation also had the advantage of allowing us to study if the same allergens existed in both parts of the fruit. For the preparation of the extracts, the raw material (fig skin or fig flesh) was cut down and homogenised, macerated in PBS buffer (50 mM phosphate buffer, 150 mM ClNa, pH 7.5) under stirring for 24 hours at 4°C, centrifuged (5500 g for 15 minutes), dialysed and frozen, and the supernatants were lyophilised.

The protein concentrations were calculated by the

Table I. Clinical characteristics of the patients

Patient No	Gender	Age (years)	Atopy	Symptoms after ingestion of figs
1	F	47	Yes	conjunctivitis, palpebral oedema
2	F	18	No	oral allergy syndrome, facial angioedema
3	F	31	Yes	oral allergy syndrome, labial oedema
4	F	24	Yes	no previous ingestion
5	F	27	Yes	no previous ingestion
6	F	16	Yes	conjunctivitis
7	F	42	No	oral allergy syndrome, generalised pruritus
8	F	32	No	anaphylaxis

Table II. Total and specific IgE levels in the eight patients studied

Patient No.	Total IgE (kU/L)	Specific IgE, fig skin (kU/L)		Specific IgE, fig flesh (kU/L)	
1	165	5,85	(class 3)	2,23	(class 2)
2	13	<0,35	(class 0)	< 0,35	(class 0)
3	484	<0,35	(class 0)	<0,35	(class 0)
4	427	8,21	(class 3)	3,53	(class 3)
5	347	13,55	(class 3)	7,14	(class 3)
6	26	0,91	(class 2)	0,64	(class 2)
7	50	0,36	(class 1)	<0,35	(class 0)
8	238	0,65	(class 1)	0,36	(class 1)

method described by Bradford⁸. The carbohydrate contents were estimated by the anthrone method⁹ in the case of the hexoses, and by the orcinol one¹⁰ for the pentoses.

The skin- and flesh-specific IgE was quantitated by enzymeimmunoassay (EIA) with BrCN-activated paper discs, using a 10 mg/ml extract concentration (0.4 mg protein/ml for the skin extract, and 0.2 mg protein/ml for the flesh one). The total IgE was assessed by a nephelometric method.

For the assessment of the protein patterns in the skin and flesh extracts we used the SDS-PAGE technique, with the discontinuous method described by Laemmli¹¹; the proteins were then stained with Coomassie blue R-250 and run together with a standard sample of proteins of known molecular masses (Pharmacia Biotech, Uppsala, Sweden). Ten- μ l samples of the problem samples or the standard were applied to each lane, each containing 20 μ g of protein as assessed by the Bradford method.

For the SDS-PAGE immunoblotting, the proteins separated in the SDS-PAGE were electrotransferred to PVDF membranes (Immobilon-P)¹² and then incubated with the patients' sera. The IgE-binding bands were developed using an enzymatic system with a peroxidase-labelled anti-IgE conjugate and then visualised with Lumigen PS-3 (chemoluminescence detection)¹³. The molecular mass standards were in this case visualised with amido black.

RESULTS

The prick-by-prick skin tests with the fresh fruit were positive in all eight patients, while the tests performed with the commercial extract were negative in all of them. The prick tests with extract of Fb leaves was positive in all eight patients, that with kiwi extract in three, that with latex in one and that with papain in another one; the skin tests with bromelain and mulberry pollen extract were negative in all patients. Among the eight patients, five were

atopic (sensitization to mites and/or pollens). The skin tests with fresh fig were negative in all ten control atopic subjects.

The oral challenge test was carried out on five patients (patient No. 2 refused), and was positive in all five. Patients Nos. 1, 6 and 7 developed symptoms identical to those that had prompted consultation. Patients Nos. 4 and 5 (who reported no previous known ingestion of figs) evidenced, 15 minutes after the ingestion, rhinoconjunctivitis and oral allergy syndrome. In all cases, the symptoms were controlled with oral antihistamines.

The total serum IgE levels ranged between 13 and 484 kU/l. The results of the enzymeimmunoassay revealed significant levels of specific IgE to the fig skin and/or flesh extracts in six of the eight patients studied (Table II), while the assessments carried out in the sera of non-allergic persons were negative.

The fig skin and flesh extracts evidenced the same carbohydrate proportions by weight (60% hexoses and 5% pentoses). However, the protein content was higher in the skin extract (4%) than in the flesh one (2%).

The electrophoresis of the fig extracts (both skin and flesh) in the presence of SDS yielded similar band patterns both in the presence and in the absence of β -mercaptoethanol, with predominant bands in the 24 - 29 kDa molecular mass range. In the absence of β -mercaptoethanol there was a high proportion of high-molecular weight proteins ($\approx$ 40 - 67 kDa) while in the presence of that agent a high percentage of low-molecular weight proteins ($\approx$ 13 - 15 kDa) was detected, reflecting the effects of the reducing agent (β -mercaptoethanol) on the possible disulfide bridges stabilising the structures of some of the proteins present in the extracts.

The pattern of IgE-binding bands observed in SDS-PAGE immunoblotting was similar for the two types of extracts (fig skin and fig flesh), both in the presence and in the absence of β -mercaptoethanol. However, when the samples

had not been treated with β -mercaptoethanol there was more pronounced IgE binding in the high-molecular weight mass range (between 70 and 50 kDa), with particularly manifest binding in a band corresponding to molecular weights $\approx$ 74/64/60 kDa and in a MW $\approx$ 16/14/13 kDa band triplet. In the case of the samples treated with β -mercaptoethanol the more salient IgE-binding bands corresponded to approximately 61, 42, 33, 25 and 17 kDa (Fig. 1).

DISCUSSION

The fig is a traditionally consumed fruit in our country; nevertheless, but few cases of food allergy triggered by the ingestion of this fruit have been reported to date. There have been however reports of phototoxic reactions after contact with figs¹⁴, and the photoactive components have been isolated and quantified¹⁵. We here report a study of eight patients in whom allergic sensitization and symptoms after ingestion of figs have been demonstrated; allergy to this fruit is thus not as infrequent as might be surmised from the very few reports published to date. This prevalence might be further increased by the ever more frequent exposure of the population to *Ficus benjamina*, used as an indoor decorative plant^{7,16,17} and with which the fig evidences cross-reactivity. In this context, it should be pointed out that all our patients were females, and this is possibly the fraction of the population that is more exposed to Fb in the home indoor environment. Atopy might be considered to represent a risk factor for sensitization, as most of the patients in our study evidenced sensitization to some of the more prevalent airborne allergens in our area.

The results of our work stress the importance of using the fresh fruit for carrying out the skin tests: while the prick tests with a commercial fig extract were negative in all our eight patients, the prick-by-prick with the flesh of fresh figs was positive in all of them. This phenomenon is quite frequent with other foodstuffs, but even more so with fruits. Because of the known cross reactivity between fig and other allergens, and also of the fact that only in about 40% of the patients with presumed food allergy is that allergy confirmed in the challenge tests¹⁸, we consider advisable to perform challenge tests on all patients unless formal contraindications exist.

The specific IgE measurements evidence a trend towards higher values with the skin extract than with the flesh one. This observation might be explained by the greater proportion of protein in the former, which might yield higher concentrations of protein bound to the cellulose disc used for the enzymeimmunoassays.

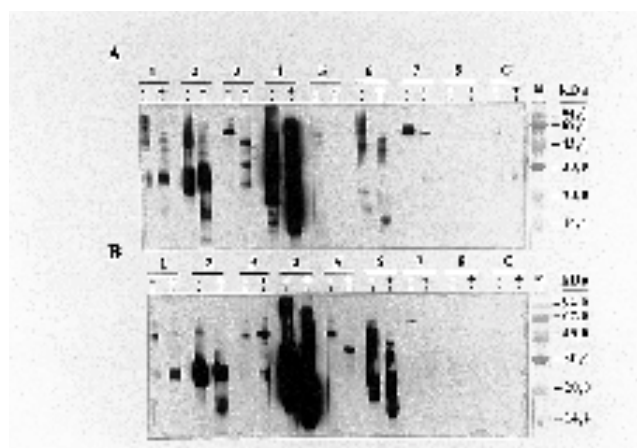


Fig. 1. SDS-PAGE immunoblotting of the fig skin and flesh extracts. A: Skin extract; B: Flesh extract. (-): Samples not treated with β -mercaptoethanol; (+): Samples treated with β -mercaptoethanol. Lane 1: patient 1; lane 2: patient 2; lane 3: patient 3; lane 4: patient 4; lane 5: patient 5; lane 6: patient 6; lane 7: patient 7; lane 8: patient 8; lane "C": control; M: molecular mass standard.

The IgE binding in the SDS-PAGE immunoblot assay occurs predominantly in the high molecular mass range when the samples had not been treated with β -mercaptoethanol, but in the low molecular mass range after treatment of the samples with this reducing agent. This fact points out the contribution of the disulfide bonds in maintaining the quaternary structure of the allergens present in the sample. This behaviour, as well as the size of the IgE-binding bands (74/64/60 kDa and 16/14/13 kDa in the absence of β -mercaptoethanol, and 42, 33, 25 and 17 kDa in the presence of the reducing agent), are similar to those previously observed for the allergens present in *Ficus benjamina*^{7,19}. All these observations and findings confirm the close similarity between the allergens present in the two raw materials, as had been previously deduced from the results of EIA inhibition techniques^{1,6}.

The lack of correspondence usually observed between the results of the skin prick tests when performed with the fresh fruit or with commercial extracts, the effects of β -mercaptoethanol treatment, and the difficulties observed in the course of our work in achieving good and easy reproducibility of the SDS-PAGE immunoblot results (intensity and clarity of the bands) are clearly indicative of the lability of the allergenic epitopes present in the fig samples.

In all patients who are allergic to figs, the eventual presence of sensitization to other taxonomically related allergens should be investigated. This is particularly true for the sap of *Ficus benjamina* and for other allergens related to both, such as kiwi, *Hevea brasiliensis* latex and papain.

REFERENCES

1. Dechamp C, Bessot JC, Pauli G, Deviller P. First report of anaphylactic reaction after fig (*Ficus carica*) ingestion. *Allergy* 1995; 50: 514-516.
2. Oei HD, Tjiook SB. A case report of an anaphylactic reaction after Fig (*Ficus carica*) ingestion. *J Allergy Clin Immunol* 1998; 10: S31.
3. Hemmer W, Focke M, Götz M, Jarisch R. Allergy to Fig (*Ficus carica*). *J Allergy Clin Immunol* 1998; 10: S93.
4. Delbourg MF, Moneret-Vautrin DA, Guilloux L, Ville G. Hypersensitivity to latex and *Ficus benjamina* allergens. *Ann Allergy Asthma Immunol* 1995; 75: 496-500.
5. Brehler R, Abrams E, Sedlmayr S. Cross-reactivity between *Ficus benjamina* (weeping fig) and natural rubber latex. *Allergy* 1998; 53: 402-406.
6. Díez-Gómez ML, Quirce S, Aragonese E, Cuevas M. Asthma caused by *Ficus benjamina* latex: evidence of cross-reactivity with fig fruit and papain. *Ann Allergy Asthma Immunol* 1998; 80: 24-30.
7. Gaig P, Bartolomé B, Enrique E, García-Ortega P, Palacios R. Hypersensitivity to *Ficus benjamina*. *Alergol Immunol Clin* 1999; 14: 212-217.
8. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein-dye-binding. *Anal Biochem* 1976; 72: 248-254.
9. Chung CW, Nickerson WJ. Polysaccharide synthesis in growing yeasts. *J Biol Chem* 1954; 208:395-406.
10. Volkin E, Cohn WE. Estimation of nucleic acids In: *Methods of Biochemical Analysis*. New York, NY, USA: Interscience Publishers, 1957; 1: 298-305.
11. Laemmli UK. Cleavage of structural proteins during the assembly of the head bacteriophage T. *Nature* 1970; 227: 680-685.
12. Towbin H, Staehelin I, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedures and some applications. *Proc Natl Acad Sci USA* 1979; 76: 4350-4354.
13. Akhavan-Tafti H, Sugioka K, Arghavani Z, DeSilva R, Handley RS, Sugioka Y, et al. Lumigen PS: Chemiluminescent Detection of Horseradish Peroxidase by Enzymatic Generation of Acridinium Esters. *Clinical Chemistry* 1995; 41: 1368-1369.
14. Ippen H. Phototoxic reaction to figs. *Hautarzt* 1982; 33: 337-339.
15. Zayoun ST, Aftimos BJ, Asi Ali L, Tenekjian KK, Khalidi U, Kurban AK. *Ficus carica*: isolation and quantification of the photoactive components. *Contact Dermatitis* 1984; 11: 21-25.
16. Axelsson IGK, Johansson SGO, Zetterström O. A new indoor allergen from a common non-flowering plant. *Allergy* 1987; 42: 604-611
17. Bircher AJ, Langauer S, Levy F, Wahl R. The allergen of *Ficus benjamina* in house dust. *Clin Exp Allergy* 1995; 25: 228-233.
18. Sampson HA. Food allergy. Part 2: Diagnosis and management. *J Allergy Clin Immunol* 1999; 103: 981-989.
19. Axelsson IGK, Johansson SGO, Zetterström. Characterization of Allergenic Components in Sap Extract from the Weeping Fig (*Ficus benjamina*). *Int Arch Appl Immunol* 1990; 91: 130-135.