
Case reports

Occupational rhinoconjunctivitis and food allergy because of aniseed sensitization

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Background: Aniseed is a spice frequently used in Mediterranean cooking and, as with other Umbelliferae, it has been involved in clinical allergy.

Objective: This investigation was undertaken to study the allergens implicated in a case of occupational allergy to aniseed associated with rhinoconjunctivitis and gastrointestinal symptoms.

Methods: Skin prick tests were performed to inhalant allergens, spices used in the patient's workplace (aniseed and cinnamon), and 12 other Umbelliferae spices, birch, and mugwort. A nasal challenge test to aniseed and cinnamon and a double-blind placebo-controlled oral food challenge test to aniseed were also performed. The molecular weights of the allergens were studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoblotting and cross-reactivity among Umbelliferae species by enzyme immunoassay inhibition.

Results: Skin prick tests showed a positive immediate response to aniseed, asparagus, caraway, coriander, cumin, dill, and fennel extracts, and an intense late response to aniseed. Skin prick tests to celery, carrot, birch pollen, and mugwort pollen extracts were negative. Results of a nasal challenge test were positive to aniseed and negative to cinnamon; an aniseed oral food challenge test yielded a positive response. The molecular weights of the main immunoglobulin (Ig)E-binding proteins in aniseed extracts were approximately 48, 42, 39, 37, 34, 33, and 20 kD. Caraway, fennel, cumin, and coriander extracts showed similar IgE-binding patterns. Enzyme immunoassay inhibition studies with the patient's serum revealed cross-reactivity among the IgE components from aniseed, caraway, coriander, fennel, and dill extracts.

Conclusions: We demonstrate the presence of aniseed allergens in a case of occupational rhinoconjunctivitis and food allergy, with molecular weights for this spice that differed from those previously reported.

Ann Allergy Asthma Immunol 2002;88:518–522.

INTRODUCTION

Umbelliferae (or Apiaceae) is an herb plant family with more than 3,000 species found in mild climate areas. They are used as food (carrot, parsnip, and celery) and seasoning (parsley, cumin,

dill, fennel, marjoram, caraway, aniseed, and coriander). This plant family has been associated with allergic reactions not only causing respiratory problems^{1,2} and food allergy,^{3,4} but also acting as an elicitor of contact allergy.^{5,6}

Some patients who have pollen-related allergy have also experienced food allergy as a consequence of pollen, fruit, and vegetable cross-reactions, such as the mugwort-birch-spices syndrome, birch pollen-Rosaceae reactions, or

mugwort pollen-Umbelliferae reactions. Although Umbelliferae vegetables, such as carrots and celery, are the most important in the mugwort-birch-spices syndrome, some spices (aniseed, cumin, caraway, coriander, dill, and fennel) have also been involved. Proteins of 14 kD (profilin), 17 kD (related to Api g 1, Bet v 1, and Cor a 1 allergens), and approximately 46 to 60 kD⁷ have been suggested as responsible for this syndrome.

Anise (*Pimpinella anisum*) is a perennial flowering plant originally from Egypt. It is grown in Mediterranean countries, and its seed is frequently used in the confectionery and meat industries, as well as in the making of drugs, perfumes, and spirits (anisette). Umbelliferae spices have been described as causing occupational asthma^{2,8} and other job-related problems, but inhalation of aniseed dust has been reported only once as a cause of occupational asthma.⁹

CASE REPORT

A 43-year-old woman, who did not smoke and had no family or personal history of allergy, reported a 4-year history of episodic symptoms of rhinoconjunctivitis associated with her workplace, where she had been employed as a baker and confectioner for 10 years. She noticed rhinorrhea, watery eyes, sneezing episodes, and itchiness of nose and eyes in her work environment when aniseed and cinnamon were used in the making of biscuits.

During the past 2 years, the patient had also noticed general malaise,

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Received for publication March 12, 2001.

Accepted for publication in revised form August 29, 2001.

drowsiness, abdominal bloating, and epigastric cramping pain, which persisted for 2 to 3 hours immediately after eating food that contained aniseed, asparagus, cumin, parsley, and marjoram, as well as precooked soups and sausages. She tolerated other spices (pepper and clove).

MATERIALS AND METHODS

Skin Tests

Skin prick tests were performed with use of standardized lancets (Dome Hollister, Leverkusen, Germany), in accordance with the recommendations of the European Academy of Allergology and Clinical Immunology.¹⁰ The patient discontinued treatment with topically and systemically administered corticosteroids 2 weeks before and antihistamines 10 days before these tests.

Skin prick tests (Bial-Aristegui, Bilbao, Spain) were performed with the most common inhalant allergens in our area (pollens, mites, molds, and danders) as well as with birch pollen (*Betula alba*), storage mites (*Acarus siro*, *Euroglyphus maynei*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Blomia kulagini*), wheat, wheat flour, rye, barley, oats, rice, corn, α -amylase, egg white, and milk.

Protein extracts from aniseed, cinnamon, carrot, celery, asparagus, cumin, parsley, marjoram, caraway, dill, fennel, coriander, pepper, and clove were prepared by magnetic stirring during agitation in phosphate-buffered solution (50 mmol/L) and NaCl (150 mmol/L) at pH 7.5 overnight at 4°C. The samples were centrifuged at 5,600 *g* for 30 minutes, and the supernatant was dialyzed against water and freeze-dried. The extracts were diluted in phenolated and glycerinated saline solution at a concentration of 20 mg/mL.

Histamine hydrochloride (10 mg/mL) and phenolated glycerol saline solution were used as positive and negative controls, respectively. Ten subjects without atopy were tested with aniseed and cinnamon as negative controls.

Determination of Immunoglobulin (Ig)E

Total IgE was measured with use of the Pharmacia CAP fluorescence enzyme immunoassay (Uppsala, Sweden). Specific IgE levels to molds (*Alternaria*, *Cladosporium*, and *Penicillium*), α -amylase, and storage mites were also determined with use of the Pharmacia CAP System. Specific IgE levels to cinnamon, carrot, celery, caraway, cumin, fennel, marjoram, coriander, asparagus, parsley, dill, birch pollen, mugwort pollen, and aniseed were obtained by coupling the extract solutions (10 mg/mL) to the 6-mm diameter cyanogen bromide-activated paper disks, as described by Ceska and Lundqvist,¹¹ and using an enzyme immunoassay kit following the manufacturer's instructions (HYTEC, Hycor Biomedical, Portland, ME).

Analytical Methods

Proteins were measured as described by Bradford,¹² hexoses by the anthrone method,¹³ and pentoses by the orcinol method.¹⁴

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in reducing conditions (5% β -mercaptoethanol) in accordance with the method of Laemmli,¹⁵ with a 12.5% separating gel and a 4% stacking gel. Proteins were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Milford, MA), essentially as described by Towbin et al.¹⁶ Immuno-detection was performed after incubation with a suitable serum dilution, by a chemiluminescent detection method following the manufacturer's instructions (ECL+Plus Western blotting detection system, Amersham-Life Science, Uppsala, Sweden). Electrophoretic results were analyzed using densitometer image analysis (BioRad Laboratories, Hercules, CA).

Enzyme Immunoassay Inhibition

Enzyme immunoassay inhibition was performed according to methods reported by Yman et al.¹⁷ Serial dilutions (0.0001, 0.001, 0.01, 0.1, and 1 mg/

mL) of all the heterologous inhibitor extracts were used for the assay.

Nasal Challenge Test

A nasal challenge test with both aniseed and cinnamon was performed. The patient had taken no medication during the previous month. Before the study, functional respiratory tests were performed following the American Thoracic Society guidelines,¹⁸ and a nasal examination ruled out any mechanical obstruction of the nostrils.

The patient was studied with use of the active anterior rhinomanometry technique, following the criteria from the Committee Report on Standardization of Rhinomanometry,^{19,20} by means of a Rhinotest MP 500 (EVG Elektronik-Vertriebs GMBH, Böhl-iggelheim, Germany).

The nasal tests were performed 1 month apart. After spraying 0.2 mL of diluent (human albumin solution, 0.03%), four increasing concentrations (0.0005, 0.005, 0.05, and 0.5 mg/mL) of allergen were sprayed into the same nostril every 15 minutes until a positive result was achieved. Ten subjects without atopy were tested as negative controls.

Oral Challenge Test

A double-blind, placebo-controlled, oral food challenge test to aniseed dust was performed.²¹ At the time of this test, the patient was free of symptoms and had not taken any antihistamines for 4 weeks. Opaque capsules prepared by the hospital pharmacy were used as vehicles, containing 1, 5, 10, 20, 40, and 80 mg of aniseed. Dextrose was used as placebo. Before the test, her blood pressure was 110/70 mm Hg, and her pulse was 74 beats/minute. Physical examination showed no abnormalities, and a forced spirometry showed a normal ventilatory pattern.

RESULTS

Skin Tests

Results of skin prick tests are shown in Table 1. A notable 64-mm² wheal occurred as an immediate response to aniseed. The late response to aniseed was also substantial, with a 601-mm²

Table 1. Results of Skin Prick Tests (Immediate and Late Responses) and of RAST for Allergens Studied*

Allergen	Skin prick test†		RAST	
	Immediate	Late‡	kU/L	Class
Aniseed	64	1,009	7.03	3
Asparagus	27	0	<0.35	0
Caraway	57	0	2.2	2
Carrot	0	0	<0.35	0
Celery	0	0	0.7	2
Cinnamon	0	0	0.6	1
Coriander	38.5	0	1.8	2
Cumin	30	0	1.0	1
Dill	41	0	<0.35	0
Fennel	41	0	2.1	2
Histamine	36	0	...	...
Parsley	0	0	<0.35	0

* RAST, radioallergosorbent test.

† Wheal area (mm²).

‡ As observed at 24 hours.

area of involvement at 6 hours and a 1,009-mm² wheal at 24 hours, disappearing at 48 hours. Immediate and late responses to cinnamon were negative. Results of skin prick tests were negative in control subjects.

Specific IgE Measurements

The total IgE level was 23 kU/L. Determination of specific IgE showed positive results to aniseed, caraway, celery, cinnamon, coriander, cumin, and fennel (Table 1). No IgE was found for the other allergens tested.

Biochemical Characterization of the Extracts and Cross-Reactivity Studies

Calculation of the protein and carbohydrate contents of the spice extracts (aniseed, caraway, coriander, cumin, and fennel) yielded the following mean values: protein content $23.7 \pm 9.7\%$ (two standard deviations; wt/wt), hexose content $20.1 \pm 7.8\%$ (wt/wt), and pentose content $10.6 \pm 6.2\%$ (wt/wt).

The protein composition of the extracts studied by SDS-PAGE showed a range from 14 to 70 kD. The molecular weights of the most predominant proteins were in the following approximate ranges: 44 to 50, 30 to 33, and 18 to 20 kD (Fig 1).

The SDS-PAGE immunoblotting of the extracts revealed IgE-binding bands of approximately 48, 42, 39, 37, 34, 33, and 20 kD in aniseed extract;

54, 42, 38, 31, and 20.8 kD in caraway extract; 66.5, 51.6, 43, 38, 32, 28, 25.8, and 19 kD in cumin extract; 57, 42, 38, 33, and 20 kD in fennel extract; and 57, 50, 39.5, 34, and 20 kD in coriander extract (Fig 1).

When cross-reactivity studies were performed with use of aniseed extract as solid phase, a clear heterologous inhibition was obtained (Fig 2).

Nasal Challenge Test

Forced spirometry showed a normal ventilatory pattern. A nasal challenge test demonstrated positive results with aniseed at the lowest concentration (0.0005 mg/mL), showing an increase in both nasal resistance (160%) and symptoms (sneezing, rhinorrhea, and

intense nasal pruritus), but no late response. Results of a nasal challenge test were negative to cinnamon at every concentration. In the 10 control subjects, results of nasal challenge tests to aniseed were negative.

Oral Challenge Test

Six minutes after the ingestion of the capsule containing 1 mg of aniseed, the patient began to yawn and to experience general malaise, epigastric cramping, and chest tightness. Her blood pressure was 100/75 mm Hg, and her heart rate was 78 beats/minute. Results of spirometry were normal. Except for intense abdominal bloating, the findings on physical examination were normal. No nasal, ocular, or respiratory symptoms were noted. Three hours later, the patient was free of symptoms.

DISCUSSION

The Umbelliferae family has been associated with various allergic conditions: anaphylaxis (coriander,³ cumin,⁴ and aniseed²²), urticaria and angioedema,²³ rhinitis and contact urticaria (bishop's weed²⁴), occupational asthma (aniseed⁹), and other disorders (skin and otolaryngeal irritation, rhinitis and asthma, oral allergy syndrome, and abdominal pain).²⁵ Cross-reactions have been described with carrot, celery, birch, and mugwort (known as the mugwort-birch-spices syndrome²⁶), and other spices,²⁷⁻²⁹ although such reactions have not previously been related to aniseed.²³

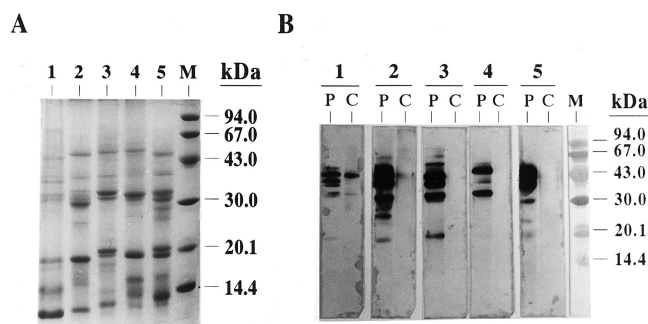


Figure 1. Electrophoretic and immunoblotting results. A) SDS-PAGE and B) SDS-PAGE immunoblotting. Lane 1: Aniseed extract. Lane 2: Cumin extract. Lane 3: Dill extract. Lane 4: Coriander extract. Lane 5: Caraway extract. Lane P: Patient serum. M: Molecular weight marker. C: Control serum (pool from nonatopic subject's sera).

In our patient, a skin prick test to *Artemisia* was negative. Interestingly, neither this plant nor birch is found in our area. We were unable to evaluate the ability of celery to induce symptoms in this patient because she does not like it, never eats it, and refused an oral challenge test with celery.

Occupational allergy, a frequent problem, has been described in relationship to various types of employment (such as bakers, animal caretakers, harbor laborers, carpenters, and health care professionals). In reference to respiratory diseases, only one previous case has been reported with immediate positive results to a skin prick test, radioallergosorbent test, and bronchial provocation, in a butcher with occupational aniseed-induced asthma,⁹ but no biochemical characterization or cross-reactivity studies were undertaken.

Although the etiologic agent of these problems has not always been identified, proteins and glycoproteins from handled material, as well as proteins from fungi growing in the workplace, have frequently proven to be the cause. In our currently described case, the patient initially had rhinoconjunctivitis at her workplace, when she was using powdered aniseed and cinnamon in the preparation of confectionery. The allergy ultimately resulted in gastric disturbance from ingestion of spices. These concurrent respiratory and gastric problems have been reported previously in some cases of occupational spice-related allergy,² with women having Umbelliferae allergy more commonly than men.²⁵

Our patient had a notable late-phase skin reaction, with an intense specific sensitization. This response has been seen with other allergens (pollens, mites, molds, danders, insulin, and enzymes), but to our knowledge, it has not been described with spices.³⁰ In addition, with only 1 mg of aniseed, the patient showed immediate digestive symptoms in the oral challenge test, compatible with food allergy in the adult.³¹ This precipitating quantity was considerably lower than those reported previously, which ranged from 0.5 to 1 g.³²

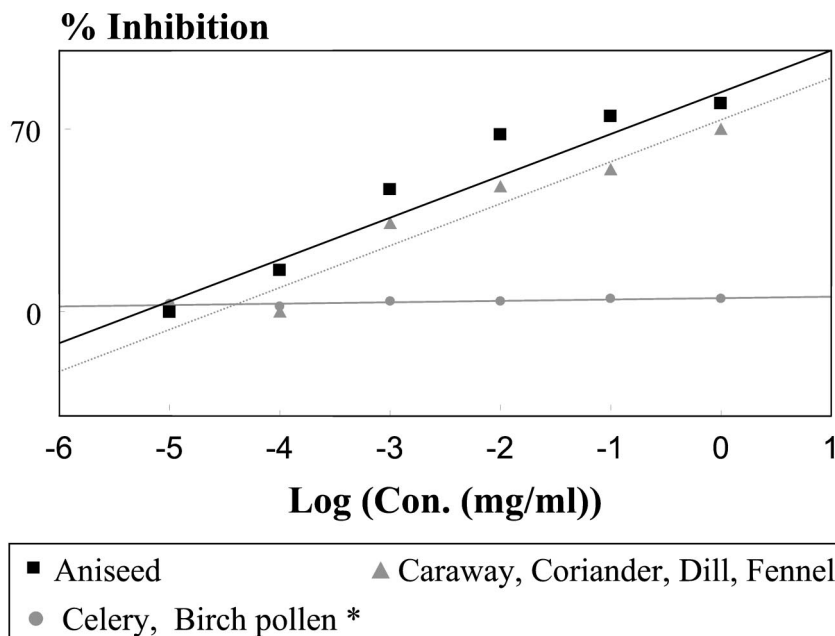


Figure 2. Results of cross-reactivity studies (enzyme immunoassay inhibition) with use of aniseed extract as solid phase. *, allergens tested separately but yielded similar results.

The molecular weights of the allergens involved in our patient were similar to those described in cases of Umbelliferae occupational allergy, but different from those previously published for the mugwort-birch-spices syndrome: 14 kD (profilin), 17 kD (Bet v 1 related), and 46 to 60 kD.^{1,7,8} The allergic reaction to precooked soups and other cooked foods demonstrates the thermal acid-resistant nature of the epitopes involved.

We demonstrated the existence of cross-reacting allergens among aniseed and other Umbelliferae spices (caraway, coriander, dill, and fennel) by in vivo and in vitro studies. These allergens seem to differ from those described by Fraj et al⁹ in the only previous report of occupational aniseed allergy, in which allergens produced respiratory problems (asthma) but no food allergy. These investigators attributed this outcome to the thermolabile nature of the allergens and the action of the digestive processes.

In our patient, the rhinoconjunctivitis crises were always related to her work environment or to spice shops where unpacked aniseed was sold. The

data presented (results of skin prick tests [immediate and late responses], IgE levels, nasal challenge test, and oral challenge test) support the fact that Umbelliferae were the cause of her nasal and digestive allergy.

CONCLUSION

Currently, our patient is free of symptoms after avoiding exposure to and ingestion of aniseed and the other Umbelliferae mentioned. Therefore, we conclude that aniseed is an allergen that should be taken into account in the diagnosis of persons with symptoms who have been exposed to it in their jobs (eg, confectioners, butchers, and those in the precooked food industry).

ACKNOWLEDGMENT

We thank Ian Johnstone for help with the English language version of the manuscript.

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