

Squid hypersensitivity: a clinical and immunologic study

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Hypersensitivity to mollusk has rarely been described in the literature. Among the mollusks, the cephalopods are a group of great importance as a food source.

We report seven patients who had had symptoms highly suggestive of IgE-mediated reactions after ingesting squid or inhaling vapors from cooking squid. All had previously suffered from persistent rhinitis or asthma for years. In addition, six of the seven patients had had symptoms after ingesting shrimp. Skin prick tests were strongly positive for boiled squid extract and for various commercial crustacean extracts. Specific IgE antibodies against boiled extract and several crustacean extracts were demonstrated in all patients by RAST and reverse enzyme immunoassay. Cross reactivity between squid and shrimp and other crustaceans was demonstrated by reverse immunoassay inhibition studies. Cross reactivity could not be demonstrated between squid and octopus, which are both cephalopods, nor between squid and other mollusks.

INTRODUCTION

Hypersensitivity reactions to foods are very common among atopic patients. The causal agents vary from region to region and according to dietary patterns.¹ Seafood often causes adverse reactions. Mollusks, especially squid, are common food sources in the Canary Islands. The squid is a member the phylum Mollusca which includes round clams, mussels, oysters, cockles, and snails. This phylum, second in importance after the Arthropods, is very diverse and widely distributed in the sea, fresh water, and on land. This group is of great importance as a food source.²

Immediate hypersensitivity to mollusks, and cephalopods in particular, has been considered exceptional though sporadic cases have been reported after ingestion of oysters, round clams, mussels, and snails.³

This study of seven patients with

symptoms highly suggestive of Type I hypersensitivity to squid demonstrated allergy to cooked squid antigen by immediate skin test reactivity, RAST, and reverse enzyme-immunoassay (REIA). In addition, cross sensitivity between squid, other mollusks, and crustaceans was investigated using inhibition studies.

MATERIAL AND METHODS

Patients

The group consisted of seven adults (four men and three women) with persistent rhinitis and/or asthma due to sensitization to house dust mites. They had also had immediate symptoms highly suggestive of allergic responses after ingesting or inhaling vapor from cooked squid. Six of the seven patients had had symptoms immediately after consuming shrimp, but none reported reactions to other mollusks (octopus, oyster, round clam, and mussel) or to crustaceans (lobsters and crab).

The clinical characteristics of the study group are shown in Table 1. Their symptoms associated with ingesting cooked squid or inhaling aerosol particles from cooking are shown in Table 2.

Antigen Extracts

Squid. Fresh squid were thoroughly washed with water and then completely dissected. The central plate was removed, then the mantle and fins were triturated and two separate extraction procedures were followed:

Raw extract. Two grams of squid meat was mixed in 20 mL of phosphate buffered saline (PBS), pH = 7.3 at laboratory temperature and the preparation was macerated for 60 minutes. Then the sample was centrifuged for ten minutes at 1,000 g. The supernatant was separated and sterilized by passing it through a millipore filter (pore size 0.22 microns) (Millipore Co, Bedford, Mass). A final concentration of 1:10 wt/vol was obtained, aliquoted, and stored at -20 °C for later use.

Boiled extract. Two grams of triturated squid flesh was macerated in 20 mL of PBS for 60 minutes, then cooked at 75 °C for 15 minutes. After cooling, it was centrifuged for ten minutes at 1,000 g and then the supernatant was separated and sterilized by passing it through a millipore filter. A final concentration of 1:10 wt/vol was obtained, aliquoted, and stored at -20 °C for later use.

Ten-fold dilutions were made for

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Table 1. Characteristics of Study Population

Patients	Age, yr	Sex*	Family History of Atopy	Family History of Perennial Rhinitis/Asthma
1	16	M	Yes	Yes
2	20	F	No	Yes
3	42	M	No	Yes
4	21	M	Yes	Yes
5	17	M	No	Yes
6	20	M	Yes	Yes
7	14	F	Yes	Yes

* M = male and F = female.

Table 2. Symptoms After Squid Ingestion or After Inhalation of Steam Arising from Boiling Squid

Symptoms	Number (Percentage)
Nasopharyngeal pruritus	3 (43%)
Rhinoconjunctivitis	4 (57%)
Asthma	4 (57%)
Nausea, vomiting, or diarrhea	3 (43%)
Urticaria and angioedema	3 (43%)

skin tests. Using Bradford's method⁴ we determined the protein concentrations, which were 1.0 mg/dL for the raw extract and 1.5 mg/dL for the cooked squid extract.

The same procedure was used in the preparation of the octopus and cooked round clam extracts.

Skin Tests

Skin prick tests were performed on the volar aspect of the forearm of each subject using a range of commercially available aeroallergens (*Dermatophagoides pteronyssinus*, cockroach, molds, pollens, and danders, Abelló SA, Madrid, Spain), common foods (egg, milk, fish, nuts, and cereals, Bencard, Worthing, UK). Histamine (10 mg/mL) responsiveness was assessed after 15 minutes, and those wheals with a longest diagonal equal to or greater than that induced by histamine were scored as positive.

Also, duplicate skin prick tests were applied on the volar aspect of the forearm of each subject using serial dilutions of the raw and cooked squid extracts, and also octopus and cooked round clam extracts. Tests were also performed with commercially available sea-

food extracts (shrimp, lobster, crab, oyster, and mussel) (Bencard).

Controls

The same skin tests were carried out using the extracts prepared in our laboratory on six atopic subjects and six normal individuals who served as controls.

In Vitro Studies

Total IgE. Test always performed using DELFIA (Pharmacia, Upsala, Sweden) according to the manufacturer's indications.

RAST. Performed according to manufacturer's instructions (Pharmacia) and using the following discs: *D. pteronyssinus*, cockroach, shrimp, lobster, mussel, crab, and squid.

REIA. Using REIA a search was made for squid-specific IgE and also for that specific to some crustaceans. This technique was performed as previously reported^{5,6} without any modification. This method detects the presence of specific IgE by first isolating serum IgE, then revealing it by means of peroxidase-labeled allergen.⁷ The technique consists of five steps: (1) coating of microtiter plates with a monospecific anti-human IgE, (2) incubation with serum, (3) incubation

with peroxidase-labeled allergen, (4) incubation with the substrate, and (5) the reaction is stopped and read at 492 nm.

Detection of specific IgE by REIA was performed for the following allergens: lobster, shrimp, crab (Bencard), and cooked squid. Labeling with peroxidase was performed as previously reported. Optimal concentrations used for each allergen were as follows: lobster (Bencard): 0.1 mL allergen and 2 mg of activated peroxidase, shrimp (Bencard): 0.1 mL allergen and 2 mg of activate peroxidase, crab (Bencard): 0.1 mL allergen and 2 mg of activate peroxidase, and cooked squid: 0.1 mL prepared extract and 3.4 mg of activate peroxidase.

In order to simplify the second part of the study, the sera from seven patients were pooled after confirming that they were similar. This pool was used for the REIA inhibition studies using different allergens.

REIA inhibition assay. This method is based on induced inhibition of REIA values when the peroxidase labeled-allergen is incubated together with different concentrations of unlabeled allergen in step 3 (see above), then continuing the assay as described.

Optical density reached in the serum pool was inhibited in the REIA for cooked squid using the following allergens: shrimp, lobster, crab, *D. pteronyssinus*, oyster, mussel, cod, trout (Bencard), cockroach (Abelló) and extracts of squid, octopus, and cooked round clam prepared in our laboratory.

Two-tenths milliliter (0.5 µg peroxidase per well) containing 25 µg of allergen at different dilutions (1:10, 1:100, 1:1000, and 1:10000) was incubated at room temperature for one hour in order to discover the concentration that caused 50% inhibition. All tests were performed in duplicate.

RESULTS

Clinical symptoms of all seven subjects are shown in Table 2. All had

had previous symptoms of persistent rhinitis and/or asthma exacerbated by contact with house dust mites over several years.

Of the seven patients, six (86%) had positive skin prick tests to *D. pteronyssinus* and cockroach (Table 3). All seven patients (100%) had strongly positive skin prick tests to cooked squid extract and only two (29%) had weakly positive reactions to raw squid extract. In two (29%) skin prick tests were borderline positive for octopus and cooked round clam extracts. Three patients (43%) had positive skin prick tests to oyster and mussel. Surprisingly all seven had positive skin prick tests to commercial extracts of shrimp, lobster, and crab. None of the control patients had any cutaneous reaction to mollusk extracts prepared in our laboratory.

Table 4 shows total IgE and RAST for the different allergens in our squid-sensitive patients. All these patients had RAST class 4 to *D. pteronyssinus* and ranged from 1 to 4 for the other allergens tested.

Table 5 shows the optical density obtained against shrimp, lobster, and cooked squid in REIA tests for the seven patients; optical densities obtained in control sera (20 nonallergic patients), and those of the parallel blanks for all the tests.

As can be seen in Table 6, when REIA for cooked squid was carried out, the greatest percentage inhibition of optical density in the serum pool was for cooked squid extract, with partial inhibition for shrimp, lobster, crab, and oyster extracts. There was no significant inhibition for cooked round clam, mussel, and octopus extracts. It is noteworthy that mussel, round clam, octopus, *D. pteronyssinus*, *Lolium perenne*, cockroach, cod, and trout extracts did not induce significant inhibition in the boiled squid REIA, even when the highest doses were tested.

DISCUSSION

Squid is a decapod cephalopod consisting of a fusiform body with a pair of a triangular caudal fins. A

Table 3. Skin Reactivity to Mollusk, Crustacean, *D. pteronyssinus* and Cockrach Extracts (in mm of diameter)

Extracts	Patients						
	1	2	3	4	5	6	7
<i>D. pteronyssinus</i>	5	6	11	4	8	2	8
Cockroach	2	3	5	3	3	4	3
Boiled squid	6	6	4	4	10	13	6
Raw squid	0	0	0	0	3	3	0
Boiled octopus	0	0	0	0	3	3	0
Boiled round clam	0	0	0	0	3	3	0
Oyster	2	5	4	2	3	0	2
Mussel	4	3	0	2	4	0	0
Shrimp	3	4	5	3	10	8	12
Lobster	5	3	5	2	4	10	7
Crab	3	3	5	4	7	13	6
Histamine (1%)	2	2	2	3	3	3	2

Table 4. Total IgE and Specific IgE Determination Against Different Allergens by RAST

	Patients						
	1	2	3	4	5	6	7
	357*	383*	600*	170*	265*	208*	312*
RAST, PRU							
<i>D. pteronyssinus</i>	>17.5	>17.5	>17.5	>17.5	>17.5	>17.5	>17.5
Cockroach	5.6	4.9	1.7	2.8	0.8	2.2	3.2
Squid	3.4	4.4	4.2	3.7	6.5	17.2	2.6
Mussel	3.1	0.9	1	0.3	1.8	3	0.8
Shrimp	17.3	14.3	4.8	9.9	3.2	16.5	3
Lobster	17.3	12.3	9	10.4	5	17.3	12
Crab	14.5	11.2	9.2	9.4	6.8	17.3	5

* IgE, IU/L.

Table 5. Specific IgE Determination Against Boiled Squid, Shrimp, Lobster, and Crab Extract by REIA (REIA value optical density at 492 nm)

Patients	Extracts			
	Boiled Squid	Shrimp	Lobster	Crab
1	1.460	1.334	1.353	1.444
2	1.300	1.053	1.451	1.451
3	1.280	1.101	1.345	1.630
4	1.600	1.320	1.758	1.820
5	1.870	1.619	1.926	>2.000
6	>2.000	>2.000	>2.000	>2.000
7	1.262	1.140	1.413	1.614
Controls (20 sera)		0.080 ± 0.020		
Blanks		0.070 ± 0.020		

transparent chitinous internal shell, called the feather, serves for muscle insertion. The head is surrounded by five pairs of tentacles. Ink-secreting glands that cloud the water allow it to escape from danger.² The most frequent species found in the Med-

iterranean and Atlantic is the common squid, *Loligo vulgaris*. It is widely served in many dishes.

This study included seven patients with histories of allergy for several years. All had had persistent rhinitis or asthma due to sensitiza-

Table 6. Results of REIA Inhibition Assay: Percent Inhibition Induced on the Serum Pool by Decreasing Amounts of Several Unlabeled Extracts

Extracts	% Inhibition			
	1/10	1/100	1/1,000	1/10,000
<i>D. pteronyssinus</i>	5.7	4.4	0	0
Cockroach	9	7	5.8	4.1
Boiled squid	86.4	80.3	65.4	54.6
Boiled octopus	15.8	10.4	8.1	8
Boiled round clam	14.4	10.7	10.9	9.6
Mussel	11.4	6.5	6.1	5.3
Oyster	49.8	31.6	13.2	13
Shrimp	60.2	41.3	15.5	8
Lobster	63.7	51.5	30.1	18
Crab	59.6	47.2	31.7	17.7
Cod	14.4	10.7	10.9	9.6
Trout	18.8	12.3	5.8	5.6
Lolium	7.5	7.8	9.8	7.8

tion to house dust mites. In addition, all patients reported symptoms after consuming or inhaling cooked squid, were highly suggestive of IgE-mediated immunologic mechanisms. Similar symptoms were described by six of the seven patients after consuming shrimp. The other patient had not consumed any type of seafood at all after the violent reaction after consuming squid.

The skin prick tests performed using commercially available extracts and those prepared in our laboratory together with the serologic studies suggest a type I hypersensitivity mechanism is responsible for the clinical symptoms reported by our patients.

Other studies of patients allergic to shrimp have shown that positive shrimp skin tests and RAST are more frequent among atopic patients.⁹ Our study confirms this. The results of the skin tests in the control group serve to rule out possible non-specific or irritant effects of our laboratory-prepared extracts.

It is noteworthy that all seven patients had positive skin prick tests to cooked squid extract but only two had borderline responses to the raw squid extract. Cooking may generate new allergens when the proteins in the original material are denatured by heat. This could explain the higher reactivity to cooked squid ex-

tract and its higher protein content (determined by Bradford's method). We did not investigate raw squid extract further, as here it is always eaten cooked, and all seven patients also reported symptoms on inhaling vapors from cooking. The relevant squid allergens are probably protein in nature, soluble, thermostable, and soluble.

RAST detected the presence of specific IgE antibodies to squid allergens and to different antigens from mollusks and crustaceans in sera provided by our seven patients. In most cases, RAST was class 3 or 4 to crustaceans (shrimp, lobster, and crab). In five patients, RAST squid was class 2 or 3 and only two patients presented class 4.

Using REIA, we found specific IgE antibodies in the patients' sera against the boiled squid, shrimp, lobster, and crab extracts.

The highest REIA inhibition was achieved using the cooked squid extract. There was also a significant inhibition of the optical density obtained by the squid REIA when different crustaceans were used (shrimp, lobster, and crab). Surprisingly, in spite of their phylogenetic proximity, there was not effective inhibition from another cephalopod, the octopus, or from other mollusks (oyster, mussel, and round clam). Nor was there effective REIA

inhibition using *D. pteronyssinus* or cockroach extracts, both arthropods and thus related, at least distantly, to crustaceans.² As was to be expected, there was no significant inhibition using cod or trout extracts.

Many studies have shown cross-reactivity between foods belonging to the same groups.^{10,11} Shrimp, lobster and crab, which are all crustaceans, share common antigenic determinants.^{12,13} The same might be expected in the phylum Mollusca, especially for members of the same class such as squid and octopus. The results of our study do not suggest this. There was no cross-reactivity between squid and octopus, both cephalopods nor between squid and other mollusks (round clam and mussel); only oyster extract produced nearly 50% inhibition. Cross-reactivity between shrimp and oyster, in different phyla and thus only distant phylogenetic relatives, has previously been reported. The results obtained in REIA inhibition studies allow us to suppose that the squid, to which our patients are sensitized, has common allergens with some crustaceans (shrimp, lobster, and crab).

Finally, it is noteworthy that seafoods from different phyla—the squid, a cephalopod, and shrimp, lobster and crab, which are crustaceans—show cross-reactivity. This suggests that similar or identical substances with allergenic properties are present in different foods. Further studies to investigate cross-reactivity between allergy-inducing seafoods should be undertaken.

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NOCTURNAL ASTHMA IMPAIRS DAYTIME COGNITIVE PERFORMANCE

To evaluate cognitive effects of nocturnal asthma the authors studied 12 adults with stable, chronic asthma who had had mean overnight falls in peak expiratory flow rate of at least 15% associated with awakening at least once each week due to coughing, wheezing, or shortness of breath during the previous 2 weeks. Each was matched for age and intellect with a normal subject. Usual medications were continued. These included inhaled beta agonists and corticosteroids and in eight ipratropium as well as cromolyn in two, theophylline in six, oral beta agonists in six, and oral corticosteroids in five.

Overnight sleep studies on two consecutive nights disclosed less sleeping time in the asthmatic subjects because they took longer to get to sleep. Daytime cognitive performance was worse in the subjects with nocturnal asthma (paced auditory serial addition, trail making A and B). Theophylline use had no significant independent influence on these variables.

These data indicate nocturnal asthma can impair sleep quality and daytime cognitive performance even when patients are receiving their usual maintenance therapy.

—RMS

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