

Grasshopper anaphylaxis in patients allergic to dust mite, cockroach, and crustaceans

Is tropomyosin the cause?



Within the last year, 2 patients presented to our Southern California allergy clinic with a history of severe anaphylactic reactions (one with loss of consciousness and the other with urticaria, laryngeal edema, and near syncope) after the initial consumption of chapulines (roasted grasshopper from Oaxaca, Mexico) despite no previous occupational or natural exposure to chapulines and no history of having ever eaten chapulines before. This is the first documented report of anaphylaxis to chapuline in the medical literature.

Patient 1 is a 43-year-old man with active allergic rhinoconjunctivitis and moderately severe persistent bronchial asthma. On 2 occasions in 2006 and 2011, this patient experienced urticaria and angioedema of the lips and tongue after eating soft-shell crab. In 2013, he ate roasted crickets and experienced urticaria, angioedema, and laryngeal edema. In December 2014 in Mexico City, he ate 2 servings of roasted chapulines (approximately 200 insects) served over a corn tortilla with guacamole. Within 15 minutes, he experienced itching and swelling of the lips and tongue, itchy skin, hives, chest tightness, and light headedness. During the next 15 minutes, after leaving the restaurant, according to his 10-year-old son who was with him, the patient lost consciousness 3 separate times approximately 1 to 2 minutes each time. The patient has no recall of these events. After the third episode, he stood up, developed abdominal pain, had a sudden urge to defecate, and had watery diarrhea. After this, the patient was taken to a facility, where he was treated by a physician with oral diphenhydramine, cortisone, and oral fluids and recovered, with the disappearance of the urticaria and angioedema during the next 24 to 36 hours without delayed reaction.

Patient 2 is a 50-year-old woman with active allergic rhinoconjunctivitis, bronchial asthma, intermittent urticaria, and moderately severe atopic dermatitis. The patient experienced 3 separate episodes of allergic reactions to crustaceans: urticaria from crab, angioedema from lobster, and urticaria, lip angioedema, and laryngeal edema after eating shrimp. The first 2 reactions were treated with oral diphenhydramine and the third at an urgent care facility with intramuscular diphenhydramine, dexamethasone, and 2.5 mg of nebulized albuterol. In May 2016, at a restaurant in Orange County, California, she ate a serving of approximately 140 chapulines cooked on a clay oven with lime, salt, and garlic. The patient had immediate onset of itching of the mouth and throat; swelling of the face, lips, and perioral tissue; generalized itching; throat swelling; difficulty swallowing; difficulty speaking; light-headedness; and near syncope. She was taken to an urgent care facility. She was treated with intramuscular diphenhydramine and dexamethasone and improved during the next 3 to 4 hours and had no subsequent delayed reactions.

Freeze-dried grasshopper (*Locusta migratoria*) and cricket (*Acheta domestica*) were purchased from Fluker's, Port Allen, Louisiana. Freshly prepared chapulines (*Sphenarium mexicanum*) were purchased from the restaurant in which this patient ate. All insects were ground with mortar and pestle to a fine consistency and weighed. Then the ground insect powder was dissolved at 1:10 (wt/vol) in saline for in vitro testing and in 50% glycerinated saline solutions for skin testing. Solutions were immediately stored at 4°C and periodically shaken during 24 hours. Solutions were centrifuged at 3,400 rpm for 40 minutes, the supernatant removed and

passed through sterile 0.22-μm syringe filters (SIM SIL, Inc, Irvine, California), and the filtered supernatant injected into sterile 10-mL vials and stored at 4°C. After informed written consent was obtained, patients and 5 nonallergic controls were skin tested by the prick puncture method. Patients and controls had greater than 10/15-mm (wheal/flare) histamine and negative saline and glycerine controls. Both patients had greater than 25/30-mm (wheal/flare) reactions to grasshopper and chapuline extract and greater than 15/20 mm (wheal/flare) reactions to cricket. All 5 controls had no positive reactions. Additional skin testing of the patients revealed greater than 20/30 mm (wheal/flare) reactions to cockroach, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, crab, shrimp, lobster, cat, and dog. There were negative skin test reactions to avocado, clam, and corn.

Specific IgE (sIgE) and total IgE were measured in both patients (Fig 1). Patient 1 had a total IgE level of 96.2 IU/mL, and patient 2 had a level greater than 2,000 IU/mL (reference range, 0.0–87 IU/mL). The patients had sIgE to cockroach (3.64 and 3.20 kU/L in patients 1 and 2, respectively), crab (0.96 and 1.91 kU/L in patients 1 and 2, respectively), and shrimp (0.46 and 11.5 kU/L in patients 1 and 2, respectively) (reference range, <0.35 kU/L). Patient 1 had low-level sIgE to *D pteronyssinus* (0.46 kU/L) but not to *D farinae* (<0.35 kU/L), whereas patient 2 had greater than 100 kU/L to both house dust mite (HDM) species. Patient 1 tested negative to lobster (<0.35 kU/L), whereas patient 2 tested positive at 2.69 kU/L. Both patients tested positive to cat and dog but negative to avocado, corn, and clam.

For sIgE inhibition, 2.5 mL of serum from patients 1 and 2 were incubated for 24 hours at 4°C with saline as a negative control, and with 20 mg of chapuline extract in 200 μL of saline. The results indicated that 200 μL of saline alone produced no inhibition, but the chapuline extract completely eliminated IgE binding to cockroach, crab, shrimp, and *D pteronyssinus* in patient 1 and reduced binding by 80% to cockroach, 50% to crab, 74% to shrimp, and 75% to lobster in patient 2. In patient 2, HDM (species) sIgE was not inhibited (possibly because of an unmeasurable level of sIgE to HDM >100 kU/mL). There was no inhibition of cat or dog sIgE in either patient, indicating that the effect of the grasshopper inhibition was specific for crustaceans, HDM, and cockroach.

SDS PAGE Immunoblot of grasshopper (FD), chapuline, and cockroach revealed a 30-kD protein to which the patients' IgE antibody bound. To identify the 30-kD protein to which the patients reacted, Western blotting¹ was performed using a monoclonal antibody-specific probe for tropomyosin. The antitropomyosin antibody bound to the same 30-kD protein in the chapuline and cockroach extract to which the patient's sera reacted (data not shown). This finding confirms that tropomyosin was the 30-kD protein that the patients' IgE bound to in chapuline and grasshopper.

Tropomyosin is a widely distributed, highly conserved protein that is instrumental in muscle contraction and other cell functions. Invertebrate (but not vertebrate) tropomyosin is highly allergenic because of shared, similar repeating epitopes, which results in cross-reactivity among distantly related sources.^{2,3}

Because the patients had never eaten chapulines previously, they could only have been sensitized to the tropomyosin by prior exposure to crustacean, HDM, and cockroach. Then when they, for the first time, ingested the chapulines, they experienced life-threatening anaphylaxis. Other patients have been described who were allergic to HDM tropomyosin who then, on the first ingestion

Substance	SV A No add	SV B 200 µl * Saline	SV C 20 mgm C/** 200 µl Saline	Range Units
IgE	96.2			0.0-87.0 IU/ml
Cockroach	3.64	3.64	<0.35	<0.35 KU/L
Crab	0.96	0.96	<0.35	<0.35 KU/L
Shrimp	0.46	0.46	<0.35	<0.35 KU/L
Lobster	<0.35	<0.35	<0.35	<0.35 KU/L
Clam	<0.35	<0.35	<0.35	<0.35 KU/L
<i>D pteronyssinus</i>	0.46	0.47	<0.35	<0.35 KU/L
<i>D farinae</i>	<0.35	<0.35	<0.35	<0.35 KU/L
Cat	22.9	22.9	23.9	<0.35 KU/L
Dog	2.52	2.52	2.07	<0.35 KU/L

Substance	GSL A No add	GSL B * 200 µl Saline	GSL C 20 mgm C/** 200 µl Saline	Range Units
IgE	>2000			0.0-87.0 IU/ml
Cockroach	3.20	3.32	0.65	<0.35 KU/L
Crab	1.91	2.04	1.03	<0.35 KU/L
Shrimp	11.30	10.60	2.94	<0.35 KU/L
Lobster	3.87	3.76	0.98	<0.35 KU/L
Clam	<0.35	<0.35	<0.35	<0.35 KU/L
<i>D pteronyssinus</i>	>100	>100	>100	<0.35 KU/L
<i>D farinae</i>	>100	>100	>100	<0.35 KU/L
Cat	34.9	34.1	35.2	<0.35 KU/L
Dog	2.85	2.70	2.99	<0.35 KU/L

* 200 µl Saline

** 20 mgm Chapuline/ 200 µl

*** kilo units/L

Figure 1. ImmunoCAP specific IgE and inhibition by chapuline.

of crustaceans or snails, experienced anaphylactic reactions.^{4,5} We do not know how the patients become sensitized (ie, inhalant/food or food/food). Both patients had similar skin test results and sIgE levels to crustaceans and cockroach, but patient 2 had much higher sIgE levels to HDM than did patient 1. Therefore, the specific source may not be as important as the total amount of sIgE to tropomyosin present.

It is not known whether the findings in these 2 patients are a rare coincidence or whether this phenomenon is more common. Allergy to grasshopper through occupational or natural sensitization or inhalation is rare,⁶ but ingestion of grasshopper (entomophagy) is practiced in 80% of the countries of the world,⁷ predominantly in Asia, the Middle East, Mexico,⁸ and California. California is known as the chapulines capital of America, where numerous Mexican restaurants serve the insects imported from Oaxaca and prepared in various fashions as meals and snacks.⁹ In addition to this widespread practice of eating chapulines as insects, chapuline flour, as well as grasshopper and cricket protein bars and shakes, is increasingly sold on the internet as an ecologically friendly high-protein food option.

In conclusion, these 2 patients constitute the first well-documented report of anaphylaxis after the initial ingestion of chapulines. Both patients had skin test sensitization to chapulines, and radioallergosorbent assay inhibition revealed a pan-allergen in chapuline, which cross-reacted with crustacean, HDM, and cockroach. Western blotting of chapuline identified this pan-allergen as tropomyosin. Although these are the first 2 case reports of anaphylaxis to chapulines, we suspect that this phenomenon is not rare. Ingestion of chapulines is widespread and increasing, and allergy to tropomyosin is common. Patients highly allergic to crustaceans, HDM, and cockroach should be made aware that eating chapulines and possibly other insects is a risk.

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