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Letter to the Editor

Anaphylaxis provoked by ingestion of hydrolyzed fish collagen probably induced by epicutaneous sensitization



Dear Editor,

Large amount of products derived from fish collagen, such as gelatin, hydrolyzed fish collagen and atelocollagen, are consumed as dietary supplement, sweets, cosmetics and moisturizers today. We report a patient with atopic dermatitis who experienced episodes of anaphylaxis after ingestion of dietary supplement or gummy candy, both of which contained the hydrolyzed fish collagen sold under a trade name “fish collagen peptide” even though it contained high molecular weight proteins. The patient started applying a moisturizer containing fish atelocollagen on her face 15 months before the first episode of anaphylaxis. We speculate that long term application of the moisturizer on impaired skin surface induced epicutaneous sensitization of fish collagen.

In May 2013, a 30-year old woman with a history of atopic dermatitis was referred for evaluation after episodes of anaphylaxis, which occurred twice after ingestion of yogurt with a dietary supplement, AC[®], sold by company A, that was made from hydrolyzed fish collagen, “fish collagen peptide”. Every episode occurred within several minutes after consuming 1 package of the dietary supplement with development of lip swelling, itching of eyes, throat and genitalia, and airway constriction. Since June 2009, the patient had started eating yogurt with the dietary supplement, AC[®]. Total IgE level was 1260 IU/ml. In May 2014, the patient returned to the clinic due to anaphylactic reaction after ingesting gummy candies. She noticed that anaphylaxis was provoked just by one particular fish collagen-containing gummy candy sold by company A, but never by several different gummy products without fish collagen. Because both AC[®] and this gummy candy are produced by the same company, we assumed that these products contain the allergen. Skin prick test was positive for the supplement, AC[®] (100 mg/ml water) and the hydrolyzed fish collagen, “fish collagen peptide”, supplied by company A (100 mg/ml water), an ingredient of the gummy candy (Table 1). In further inquiry, the patient disclosed that, in February 2012 when facial dermatitis worsened, she had started applying a moisturizer sold by company E containing fish atelocollagen, which is fish triple helix collagen of molecular weight of 350 kDa, composed of three alpha chains with molecular weights of approximately 110–120 kDa. Skin prick test responses were also positive for the moisturizer, fish atelocollagen supplied by company E and fish gelatin supplied by company D, but negative for several samples of gelatin derived from porcine skin or bovine bone (Table 1). The

patient denied episodes of systemic anaphylactic reaction after eating raw and cooked fish.

Analyses by SDS-PAGE and IgE western blotting showed that the patient's serum reacted with ~140 kDa protein of fish atelocollagen and 120 kDa protein of gelatin from fish collagen, and weakly with gelatin derived from bovine bone, but not with proteins of gelatin from porcine skin nor hydrolyzed fish collagen, “fish collagen peptide”, supplied by company A (Fig. 1a). IgE western blot inhibition assay revealed that the patient's serum pre-incubated with “fish collagen peptide” lost reactivity with the ~140 kDa protein of fish atelocollagen and 120 kDa protein of gelatin from fish (Fig. 1b), suggesting that ~140 kDa and ~120 kDa proteins might be cross-reactive allergens between fish gelatin and “fish collagen peptide”. The levels of specific IgE antibodies by Immuno-CAP specific IgE (Phadia Inc., Tokyo, Japan) were: 21.2 U_A/ml (class 4) for gelatin from cold water fish skin, 4.97 U_A/ml (class 3) for bovine gelatin, and below 0.1 U_A/ml (class 0) for both carp parvalbumin (Cyp c 1) and cod parvalbumin (Gdc c 1). The ~140 kDa and 120 kDa protein were analyzed by Liquid Chromatography-Tandem Mass Spectrometry (TripleTOF[®] 5600+ System, AB SCIEX, MA, USA). For identification, a database with protein sequences of *Neopterygii* extracted from the National Center for Biotechnology Information was established. The 120 kDa protein was identified to be $\alpha 2$ chain of type I collagen of *Oreochromis niloticus* (Nile tilapia) (RefSeq accession no. NP_001269826), by peptide mass fingerprinting, however ~140 kDa protein could not be identified. It is possible that this ~140 kDa protein was modified by $\alpha 2$ chain of type I collagen during processing.

In early studies, the allergenicity of fish collagen was evaluated to be low.¹ Sakaguchi *et al.* reported that some fish-sensitive patients possessed IgE antibodies against $\alpha 1$ and $\alpha 2$ chains of type I collagen.² Kuehn *et al.* reported a 12-year-old boy with hay fever and asthma who developed anaphylaxis by ingestion of marshmallows containing fish gelatin.³ Lack proposed the “dual allergen exposure hypothesis” that sensitization to allergen occurs through environmental exposure to allergen through the skin and that consumption of food allergen induces oral tolerance.⁴ Indeed, numerous cases of wheat allergy that developed from epicutaneous sensitization to hydrolyzed wheat protein (Glupearl 19S) in facial soap have been reported in Japan.⁵ Recently, cases of fish allergy induced by epicutaneous sensitization were reported.^{6,7} As the skin barrier function of majority of these patients was impaired by atopic dermatitis or hand eczema, it was estimated that direct touch with bare hands with fish meat in daily work might facilitate the sensitization to fish in these patients.^{6,7} Our patient had started applying a moisturizer containing fish atelocollagen on her face 15 months before the first episode of anaphylaxis. It has been reported that fish

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Table 1
Skin prick test.

Sample	Manufacturer	Skin prick test wheal/flare (mm)
Dietary supplement (AC [®])	A	21/25
Hydrolyzed fish collagen (ingredient of the gummy)	A	16/19
Moisturizer	E	13/13
Fish atelocollagen	E	14/16
Gelatin from porcine skin	D	0/3
Gelatin from bovine bone	D	0/3
Gelatin from fish	D	20/25
Histamine (10 mg/ml)		9/15
Saline		0/3

The size of the wheal and flare are measured after 15 min of prick test using the following samples. Dietary supplement (AC[®]) sold by company A (100 mg/ml), hydrolyzed fish collagen supplied by company A (100 mg/ml), moisturizer sold by company E (as is), fish atelocollagen supplied by company E (0.26%: concentration same as moisturizer), gelatin from porcine skin, bovine bone, fish supplied by company D (3% each), histamine dihydrochloride (10 mg/ml), and saline.

peptide during processing. Therefore, we speculate that fish atelocollagen with 350 kDa of mass was degraded by proteases to the smaller peptides on skin surface and they induced sensitization. However, the possibility cannot be excluded that the intact collagen or its degradation products with >4.5 kDa were antigens because the patient had impaired skin.

Gelatin is one of the primary components of food, medicine, cosmetics and vaccines and has been reported to cause various allergic symptoms, including anaphylaxis after ingestion of gummy bears induced by specific IgE to porcine or bovine gelatin.⁹ In a girl with episodes of anaphylaxis after ingestion of Gummy Bears, CAP-RAST test results of bovine and porcine gelatin were negative but specific IgE to fish collagen is not examined.¹⁰ In our study, we identified $\alpha 2$ chain of fish type I collagen as a causative antigen. Our case illustrates that skin external preparations containing fish atelocollagen (or processed fish collagen) should not be used for impaired skin surface of patients with atopic dermatitis to avoid the risk of epicutaneous sensitization of fish collagen.

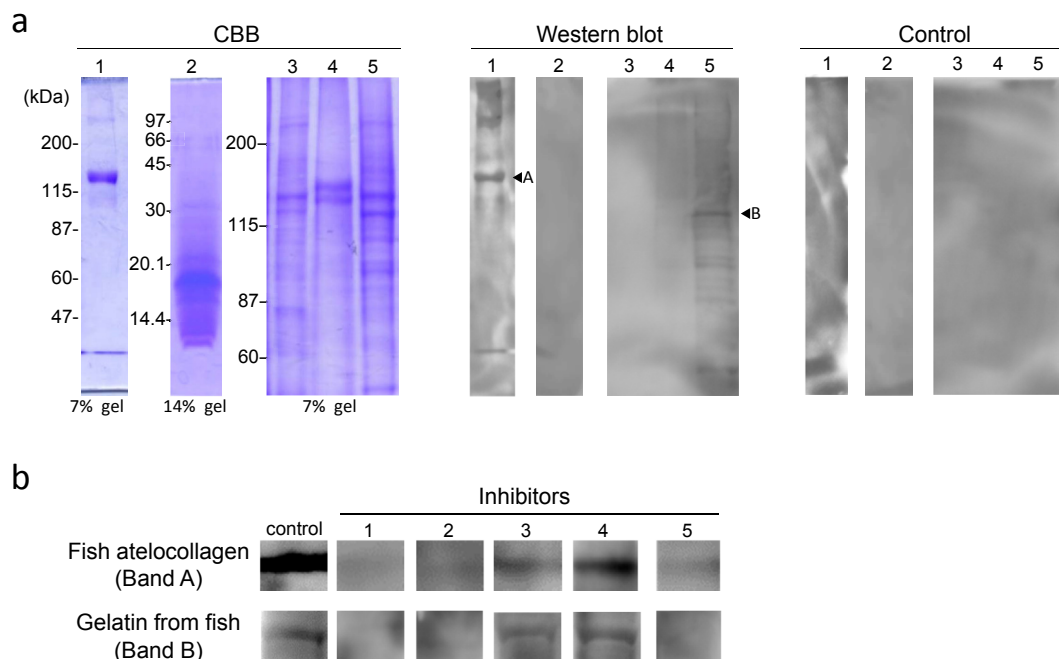


Fig. 1. IgE western blotting and inhibition assay. **a.** Each sample of collagen and gelatin: fish atelocollagen supplied by company E (lane 1), hydrolyzed fish collagen supplied by company A (lane 2), gelatins from porcine skin (lane 3), from bovine bone (lane 4), from fish (lane 5), supplied by company D, was denatured by heat at 100 °C and was electrophoresed in 7% or 14% gel SDS-PAGE. The blotted membrane was incubated with 10% patient serum, anti-human IgE (Phadia, Immuno CAP), followed by anti-mouse IgG-HRP (Cell Signaling Technology). Bound IgE were detected by a chemiluminescent reaction. IgE from the patient's serum reacted with ~140 kDa protein of fish atelocollagen (band A) and 120 kDa protein of gelatin from fish (band B). Weak reactivity was observed in proteins of gelatin derived from bovine collagen, but not with proteins of gelatin derived from porcine skin nor hydrolyzed fish collagen. **b.** Inhibition of IgE-binding to the antigens (band A and B in Fig. 1a) with the collagen and gelatin samples. Serum from the patient was preincubated with or without each inhibitors, fish atelocollagen (20 µg/ml, lane 1), hydrolyzed fish collagen (100 µg/ml, lane 2), gelatins from porcine skin (100 µg/ml, lane 3), from bovine bone (100 µg/ml, lane 4) and from fish (100 µg/ml, lane 5), for two hours at 4 °C before blotting. Hydrolyzed fish collagen inhibited the IgE-binding to the band A of fish atelocollagen and band B of gelatin from fish (lane 2). The IgE-binding to band A and B were also inhibited by gelatin from fish and fish atelocollagen, respectively (lanes 5 and 1).

collagen peptides of molecular weight of 3.5–4.5 kDa could highly penetrate the stratum corneum in nude mouse skin.⁸ Atelocollagen is a collagen solubilized by proteases, enzymes that break the telopeptides existed at both ends of collagen strand for crosslinking between tri-helix collagen molecules. The process of hydrolysis involving breaking down the molecular bonds between individual collagen strands and peptides by combinations of physical, chemical or biological means is used for producing collagen peptide but not for atelocollagen. Thus, atelocollagen does not usually yield small

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Conflicts of interest

AT is an employee of Phadia K.K. Thermofisher Scientific, Co., Ltd. The rest of the authors have no conflict of interest.

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