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INVESTIGATIVE REPORT

Limited Influence of Aspirin Intake on Mast Cell Activation in Patients with Food-dependent Exercise-induced Anaphylaxis: Comparison Using Skin Prick and Histamine Release Tests

Atsushi FUKUNAGA[1], Hideki SHIMIZU[1], Mami TANAKA[1], Ayuko KIKUZAWA[1], Mariko TSUJIMOTO[1], Akiko SEKIMUKAI[1], Junji YAMASHITA[1], Tatsuya HORIKAWA[2] and Chikako NISHIGORI[1]

[1]Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, and [2]Department of Dermatology, Nishi-Kobe Medical Center, Kobe, Japan

Food-dependent exercise-induced anaphylaxis (FDEIA) is a severe systemic syndrome induced by physical exercise after ingesting causative food. Aspirin is a well-known trigger for anaphylaxis in patients with FDEIA. Possible mechanisms by which symptoms are aggravated by aspirin include enhanced antigen absorption and mast cell activation. The aim of this study was to determine whether aspirin intake has an influence on mast cell/basophil activation in patients with FDEIA. Provocation tests revealed that adding aspirin to the causative food challenge in 7 of 9 (77.8%) patients with FDEIA provoked symptoms. In most cases, pretreatment with aspirin did not enhance skin tests (71.4%) or histamine release tests (88.9%) with food allergen challenges. The study confirmes that histamine release and skin prick tests can be adjunctive tools for diagnosing FDEIA. In addition, our results suggest that exacerbation of FDEIA symptoms by aspirin is not mediated by direct effects of aspirin on mast cell/basophil activation. ***Key words: food-dependent exercise-induced anaphylaxis; aspirin; histamine release test; skin test; mast cells; basophils.***

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Atsushi Fukunaga, Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. E-mail: atsushi@med.kobe-u.ac.jp

Food-dependent exercise-induced anaphylaxis (FDEIA) is a unique form of food allergy induced by exercise after food ingestion (1–4). Although the symptoms of FDEIA and cholinergic urticaria occur after exercise, FDEIA is clearly distinguishable from cholinergic urticaria, which appears as pinpoint-sized wheals with surrounding erythema that occur after sweating (5, 6). Patients with FDEIA usually have immunoglobulin E (IgE) antibodies and positive skin prick tests (SPTs) against the causative food allergens. Thus, the reactions in patients with FDEIA are generally thought to be IgE-mediated hypersensitivity to food allergens (7–10).

The causative foods are mainly wheat products and shellfish in Japan (11) and vegetables in European countries (8). Triggering factors for FDEIA include foods, exercise (12), the patient's general condition, the menstrual cycle (9, 13), and medications such as aspirin and non-steroidal anti-inflammatory drugs (14).

In particular, aspirin has been well demonstrated to induce or aggravate symptoms in patients with FDEIA (10, 15, 16). Several studies have reported that the SPT reaction to causative food allergens is enhanced by pretreatment with oral aspirin, which suggests that aspirin accelerates histamine release from mast cells and basophils, manifesting as an immediate hypersensitivity reaction (15, 17, 18). In contrast, another study demonstrated that, in patients with wheat-dependent exercise-induced anaphylaxis, aspirin uptake induced a marked increase in serum gliadin levels with the accompanying symptoms, which suggests that aspirin may up-regulate antigen uptake across the intestinal epithelium (16, 19). these two mechanisms have been proposed to account for the induction or exacerbation of symptoms by aspirin in patients with FDEIA. The aim of this study was to determine whether aspirin intake has an influence on mast cell/basophil activation in patients with FDEIA.

We performed skin tests and histamine release (from basophils) tests in 10 patients diagnosed with FDEIA, exposing them to causative food allergens by means of provocation tests before and after aspirin uptake. Neither test showed significant differences before or after aspirin uptake in most of the patients with FDEIA. These data support the opinion that aspirin intake enhances antigen uptake across the intestinal epithelium, but not activation of mast cells/basophils in most types of FDEIA.

MATERIALS AND METHODS

Patients

Six female and four male patients (ages 17–72 years) with FDEIA were enrolled in the study (Table I). All patients had a clinical history of exercise-induced anaphylaxis, especially with prior food intake. Two of the patients had allergic rhinitis. Oral and written informed consent for the study was obtained from all subjects.

Relevant drugs, such as histamine H_1-receptor antagonists, were withdrawn for at least 48 h before the trials. FDEIA was confirmed by provocation tests with the suspected foods,

Table I. *Clinical features of patients with food-dependent exercise-induced anaphylaxis*

Pat. no.	Age, years/sex	Responsible food	Total IgE IU/ml	Symptoms	Atopic diseases
1	66/F	Wheat	134	U, S	None
2	17/M	Spinach	634	U, D	AR
3	45/F	Wheat	75	U, S, A	None
4	34/F	Wheat	341	U, S	None
5	56/M	Wheat	126	U, S	None
6	53/M	Wheat	2,071	U, S	None
7	32/F	Wheat	37	U, D, A	None
8	17/F	Shrimp	991	U, D, A	AR
9	57/M	Shrimp	165	U, S	None
10	42/F	Wheat	450	U, S, D, A	None

U: urticaria; S: shock; D: dyspnoea; A: angioedema; AR: allergic rhinitis.

providing a definitive diagnosis. Cholinergic urticaria and exercise-induced anaphylaxis were ruled out because of the lack of symptoms after exercise alone. The responsible foods in patients with FDEIA include wheat, shrimp, and spinach.

Provocation tests

Provocation tests included challenges with the suspected foods, exercise, aspirin intake, and combinations of these challenges. For the wheat challenge, one piece of bread made from wheat flour (66 g) and a small amount of salt, was prepared (16). For the shrimp challenge, one fried shrimp was allotted to each patient. For the spinach challenge, 100 g of boiled spinach was prepared. Treadmill exercise was performed for 30 min after food ingestion using a slightly modified form of the protocol described by Bruce et al. (20). Aspirin (500 mg) was administered just before food intake, except in patient 4, who presented with aspirin-intolerant urticaria. When urticaria or other symptoms appeared, an antihistamine or adrenalin was given immediately.

Skin testing

Skin prick testing was performed with commercial food extracts (1:10 w/v) (Torii Pharmaceutical, Tokyo, Japan), gluten, and gliadin (1:10 w/v) (Wako, Osaka, Japan), using prick lancets (AB Nordic Medifield, Stockholm, Sweden). Reactions were read as the longest length × the perpendicular length of the wheal at 15 min. The responses were then compared with those of the positive histamine controls (10 mg/ml) and were scored as follows: 0, no positive area compared with that of the wheal on the histamine-positive control; 1+, 25%; 2+, 50%; 3+, >100%. At 2 h after aspirin intake, the SPT was performed again by the same person. The reaction to the positive histamine control was not enhanced after aspirin intake, except in patient 8.

Histamine release tests and IgE measurement

Histamine release tests were performed *in vitro* using the histamine release test (HRT) (Shionogi, Osaka, Japan) as described previously (5). Briefly, 20 µl peripheral venous blood samples from the patients and anti-basophil antibodies (BA312) conjugated with magnetic beads were added to each well of 96-well plates. They were incubated for 10 min at room temperature on a plate mixer. Antibody-binding basophils in each well were then trapped with a chandelier-shaped magnet and transferred to another microplate, where the basophils were stimulated at 37ºC for 1 h with a diluted extract of a food allergen (Torii Pharmaceutical), anti-IgE antibody (21), and digitonin, respectively. Histamine released into the medium was measured using an enzyme-linked immunosorbent assay (ELISA) with a characteristic detection profile. The mean values and standard deviation (mean ± SD; $n = 3$) of the percent histamine release from basophils of patients with the respective dilutions of commercial food extracts are shown in Table SI (available at: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1210). The statistical significance of differences between means was determined using Student's *t*-test.

Blood samples for the HRT after aspirin intake were collected 2 h after aspirin administration. When the histamine release was more than 20%, it was deemed a positive reaction. Total IgE levels and specific IgE levels were determined by fluoroenzymeimmunoassay (FEIA) methods. The cut-off value for the CAP-FEIA was set at 0.35 U_A/ml. Values < 0.34 U_A/ml were recorded as class 0 (negative); class 1 was 0.35–0.69 U_A/ml; class 2 was 0.70–3.49 U_A/ml; class 3 was 3.50–17.49 U_A/ml; and class 4 was 17.50–49.99 U_A/ml. Classes 2, 3, and 4 were deemed positive.

RESULTS

Provocation tests

All patients had a clinical history of exercise-induced anaphylaxis, especially with prior food intake (Table I). Elevated total IgE (> 270 IU/ml) levels were observed in five of the 10 patients (Table I). The results of the challenge tests are shown in Table II. Neither intake of foods alone nor exercise alone induced symptoms in any of the patients. In patient 4, aspirin intake alone provoked urticaria, suggesting that this patient also had aspirin-intolerant urticaria (22). In 4 patients (numbers 1, 3, 4, 9), ingestion of food followed by exercising resulted in urticaria. In 3 patients (numbers 3, 5, 7), ingestion of food after aspirin intake provoked urticaria. In patients 2, 6, 8, and 10, the combination of food intake, aspirin, and exercise caused urticaria, angioedema, or dyspnoea, whereas the combinations of food and exercise or food and aspirin did not provoke symptoms. Thus, adding aspirin to the causative food challenge in seven of nine patients with FDEIA provoked urticarial or upper respiratory symptoms.

Table II. *Results of provocation tests for patients with food-dependent exercise-induced anaphylaxis after 15 min exercise*

			Symptoms			
Pat. no.	Food	Aspirin, mg	Aspirin alone	Food + Exercise[a]	Food + Aspirin	Food + Aspirin + Exercise
1	Bread	500	None	U	None	Not done
2	Spinach	500	None	None	None	U, A
3	Bread	500	None	U	A, D	Not done
4	Bread	100	U	U, P	Not done	Not done
5	Bread	500	None	None	U	Not done
6	Bread	500	None	None	None	U
7	Bread	500	None	None	U, E	Not done
8	Shrimp	500	None	None	None	U, D
9	Shrimp	500	None	U, E	None	Not done
10	Bread	500	None	None	None	U, P, A

[a]No provocation for food alone or exercise alone.
U: urticaria; A: angioedema; P: palpitation; E: erythema; D: dyspnoea.

These provocation tests confirmed that all 10 patients had FDEIA.

Skin testing, histamine release tests, and IgE measurement

To verify the effects of aspirin on accelerating histamine release from mast cells/basophils, we carried out SPTs and *in vitro* HRTs (release from basophils) prior to and after aspirin intake (Table SI). Specific IgE antibodies against the causative food allergens were detected in nine of the 10 patients with FDEIA in the study. The SPTs with the causative allergens were not enhanced after aspirin intake in patients 1, 3, 6, 8, or 10. SPTs with gluten and bread were enhanced after aspirin intake in patients 5 and 7. Hence, the skin tests with causative food allergens were not enhanced by pretreatment with aspirin in 5 of the 7 patients tested. Administration of aspirin just before food intake, but not exercise after food intake, induced FDEIA symptoms only in patients 5 and 7, in whom the skin tests were enhanced.

HRTs with causative food allergen extracts were performed in all 10 patients with FDEIA prior to and after aspirin intake. Because anti-IgE antibodies could not induce enough histamine release from the basophils in patient 10, she was deemed a non-responder, and hence the HRT could not be assessed. There was significant histamine release from the basophils of all patients except patient 10 with each causative food allergen extract before aspirin intake. Histamine release with the causative food allergen did not increase significantly after aspirin intake compared with that before aspirin intake in 8 of 9 patients. In patient 6, histamine release was increased by the bread extract at a dilution of 1:1000, but at 1:10 and 1:100 dilutions the response was deemed negative and the increase was not significant.

DISCUSSION

Ingestion of a suspected food alone did not provoke symptoms in any of the patients in this study. In 4 of the 10 (40%) patients, the combination of food and exercise did induce symptoms. In contrast, the addition of aspirin induced positive provocation tests in 7 of the 9 patients tested. Similar to previous reports (15), the combination of food and exercise alone was not enough to diagnose FDEIA using provocation tests. Aspirin, a definitive factor for aggravating symptoms in patients with FDEIA, may therefore be a powerful tool for establishing a precise diagnosis of FDEIA. In contrast, the independent addition of exercise or aspirin to the food challenge induced symptoms only in patient 3. Thus, the aggravation factors may play different roles and functions in patients with FDEIA depending on the individual patient.

Few reports have documented the usefulness of HRTs in diagnosing FDEIA (15, 23). Currently, HRTs with basophils from a patient are not commonly used to diagnose FDEIA. In the present study, HRTs with an appropriately diluted extract of the particular food allergen were positive in all of the patients with FDEIA. The respective food allergen extract was used as an appropriate antigen for the HRTs because most of the extracts elicited positive skin tests. When the dilution of the extract was inappropriate (too concentrated, or too diluted), suitable results were not acquired with the HRTs (data not shown). Remarkably, HRT but not SPT for shrimp was positive in patient 9. This result shows that appropriately diluted food extracts can make the HRT a useful adjunct in the diagnosis of FDEIA. Also, specific IgE antibodies against ω-5-gliadin were detected in all of the five patients with FDEIA in this study in whom wheat was the culprit, confirming the importance of specific IgE antibodies against ω-5-gliadin in the diagnosis of wheat-induced FDEIA (WDEIA) (24).

Triggering factors for FDEIA are varied and include causative foods, exercise intensity (12), general fatigue, alcohol ingestion, cold temperature, the menstrual cycle, and aspirin (9, 10, 13, 16, 25–27). the mechanism responsible for the symptoms of FDEIA remains unclear. Concerning the role of exercise, it has been reported that increased blood levels of gliadin correlate with clinical symptoms induced by exercise in patients with WDEIA (16, 19).

Leakage from the gastrointestinal tract into the circulation was strongly induced by acute exercise in mice sensitized with gliadin and glutenin as allergens (28). Among the triggering factors, aspirin intake, especially, has been investigated in depth and reported regarding its role in provoking severe symptoms or in aggravating the symptoms of FDEIA (9, 10, 15).

Two possible mechanisms for the aspirin-induced symptoms in patients with FDEIA have been documented: up-regulation of antigen absorption across the intestinal epithelium and activation of mast cells (14–16). The latter possibility is supported by only a single previous report that SPTs with a causative food allergen were enhanced by pretreatment with oral aspirin in 5 of 8 patients with FDEIA (15). In our study, skin tests with the causative food allergen were stabilized by pretreatment with aspirin in 5 of the 7 patients tested. HRT with the causative food allergen did not increase significantly after aspirin intake in 8 of the 9 patients.

In summary, *in vivo* SPTs and *in vitro* HRTs did not show significant differences before or after aspirin intake in most of the patients with FDEIA, suggesting that the main mechanism for exacerbation of FDEIA symptoms by aspirin intake is independent of the acceleration of histamine release from mast cells/basophils. These data may support the hypothesis that antigen absorption plays a major role in the aspirin-induced aggravation of symptoms in FDEIA.

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