Using a gluten oral food challenge protocol to improve diagnosis of wheat-dependent exercise-induced anaphylaxis

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Background: Oral wheat plus cofactors challenge tests in patients with wheat-dependent exercise-induced anaphylaxis (WDEIA) produce unreliable results.

Objective: We sought to confirm WDEIA diagnosis by using oral gluten flour plus cofactors challenge, to determine the amount of gluten required to elicit symptoms, and to correlate these results with plasma gliadin levels, gastrointestinal permeability, and allergologic parameters.

Methods: Sixteen of 34 patients with a history of WDEIA and ω5-gliadin IgE underwent prospective oral challenge tests with gluten with or without cofactors until objective symptoms developed. Gluten reaction threshold levels, plasma gliadin concentrations, gastrointestinal permeability, sensitivities and specificities for skin prick tests, and specific IgE levels were ascertained in patients and 38 control subjects.

Results: In 16 of 16 patients (8 female and 8 male patients; age, 23-76 years), WDEIA was confirmed by challenges with gluten alone (n = 4) or gluten plus cofactors (n = 12), including 4 patients with previous negative wheat challenge results. Higher gluten doses or acetylsalicylic acid (ASA) plus alcohol instead of physical exercise were cofactors in 2 retested patients. The cofactors ASA plus alcohol and exercise increased plasma gliadin levels ($P < .03$). Positive challenge results developed after a variable period of time at peak or when the plateau plasma gliadin level was attained. Positive plasma gliadin threshold levels differed by greater than 100-fold and ranged from 15 to 2111 pg/mL (median, 628 pg/mL). The clinical history, IgE gliadin level, and baseline gastrointestinal level were not predictive of the outcomes of the challenge tests. The challenge-confirmed sensitivity and specificity of gluten skin prick tests was 100% and 96%, respectively.

Conclusion: Oral challenge with gluten alone or along with ASA and alcohol is a sensitive and specific test for the diagnosis of WDEIA. Exercise is not an essential trigger for the onset of symptoms in patients with WDEIA. (J Allergy Clin Immunol 2015;135:977-84.)

Key words: *Wheat-dependent exercise-induced anaphylaxis, omega-5-gliadin, gluten, anaphylaxis, cofactors, plasma gliadin levels, threshold levels, oral challenge test*

Foods, insect stings, and medications are recognized causes of anaphylaxis.[1,2] In the literature anaphylaxis in up to 59% of patients has been reported as idiopathic, although cofactor-augmented anaphylaxis has not been excluded.[3] Wheat-dependent exercise-induced anaphylaxis (WDEIA) is the best-studied model of "cofactor-induced" anaphylaxis.[1,4] Such cases are likely to be underdiagnosed.[1] Early signs and symptoms are pruritus and urticaria developing within a few minutes of exercise, which can progress to dyspnea and hypotension. The major allergen responsible is ω5-gliadin[4,5] in the gluten fraction of wheat. Exercise, acetylsalicylic acid (ASA), and alcohol are augmentation cofactors required to elicit symptoms in patients who otherwise tolerate gluten-containing products when given alone.[6-8] The disease has been classified as a subtype of exercise-induced anaphylaxis.[1] The precise mechanism remains unclear.[6]

Confirmation of WDEIA is challenging.[9] Results of skin tests and measurement of specific IgE to wheat might be negative, probably because of the low concentration of the responsible peptide in wheat flour.[6,10] Specific IgE antibodies to ω5-gliadin have been reported to have a good sensitivity (78% to 82%) and specificity (100%) for patients with WDEIA.[5,6,11,12] Challenge tests are the gold standard to confirm the clinical significance of a sensitization. In our experience and in the literature, for unknown reasons, challenge test results with wheat and exercise often are negative in patients with WDEIA despite a clear history.[13-16]

We present the first study on the use of gluten in the diagnosis of WDEIA. The goal of our study was to use escalating gluten doses with or without cofactor challenges for reproduction of symptoms and to determine clinical reaction thresholds in all patients. As will be shown, high doses of pure gluten-flour bread were able to overcome the nonresponsiveness to wheat products, such that exercise was not obligatory in many cases. Threshold plasma gliadin concentrations varied greatly between challenges but had consistently reached high levels when symptoms occurred. The sensitivities and specificities of gluten skin prick tests (SPTs) and specific IgE measurements to wheat protein fractions were determined.

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Abbreviations used
ASA: Acetylsalicylic acid
SPT: Skin prick test
WDEIA: Wheat-dependent exercise-induced anaphylaxis

METHODS

Patients

Thirty-four patients (17 male and 17 female patients; age, 23-76 years; mean age, 49 years) attending the Department of Dermatology and Allergy Biederstein between January 1, 2006, to January 31, 2013, with a clinical history of WDEIA (symptoms after exercise within 4 hours of ingestion of wheat-based products and positive ω5-gliadin–specific IgE levels [≥0.35 kU_A/L; Thermo Fisher Scientific, Uppsala, Sweden] but without chronic spontaneous urticaria or IgE-mediated wheat allergy) were recruited for the study (Fig 1). Patients' characteristics are shown in Table E1 in this article's Online Repository at www.jacionline.org. Sixteen patients (Table I) were prospectively challenged with gluten with or without cofactors: 4 patients with previous negative oral challenge results to wheat rolls or bread plus treadmill exercise, 1 patient with a previous positive challenge test result with wheat plus cofactors,[17] and 11 new patients (Fig 1). The rates of positive and negative SPT responses to gluten and specific IgE antibodies to wheat protein fractions were determined in an additional 39 consecutive adults attending the allergy department (13 male and 26 female patients; age, 18-86 years; mean age, 46 years) who underwent SPTs to wheat and gluten and had measurement of specific IgE to ω5-gliadin performed as part of their work-up for the exclusion of food allergy and served as patient control subjects (see Table E2 in this article's Online Repository at www.jacionline.org). In 8 healthy control subjects (3 male and 5 female subjects; age, 26-74 years; median age, 45 years) gastrointestinal permeability was determined before and after physical exercise (60 minutes of jogging), before and after intake of ASA, and/or before and after gluten intake. The study was approved by the Technische Universität München's Institutional Review Board, and informed consent was obtained.

Gluten flour

Pure gluten flour, which is produced by removing the water-soluble proteins from wheat flour with normal saline buffer, was obtained from Jean Pütz Products (Cologne, Germany). The ω5-gliadin concentration in the test gluten flour was determined by using HPLC and was 35 μg of ω5-gliadin/mg gluten flour.

Challenge protocol

The full study protocol takes place over 6 days (Fig 2) but can be shortened on review of the patient's past history and test results. Seven days before challenge, patients were instructed to discontinue all antiallergic drugs. After a 12-hour overnight fast, patients were given increasing amounts of bread baked with 10 to 80 g of pure gluten flour. Some patients, depending on their history and previous test results, were given increasing doses of cofactors (500-1000 mg of ASA and 10-30 mL of 95% ethanol; Braun, Melsungen, Germany) diluted with 200 mL of black currant–flavored water. The cofactors were administered 30 minutes before gluten challenge. Exercise, which was standardized to 45 minutes of aerobic exercise followed by 8 minutes of anaerobic exercise (80% and 115% of anaerobic threshold, respectively), was undertaken 30 to 60 minutes after gluten ingestion according to the typical time interval in the patients' histories. Outcomes were recorded as the dose-eliciting objective symptoms requiring discontinuation of the challenge and treatment. In 3 cases patients with positive reactions asked to be rechallenged with other cofactors (n = 1) or increased doses of gluten (n = 2) to determine their individual threshold levels for a reaction. Patients were challenged with an increasing dose of up to 1000 mg of ASA and alcohol (day 2) and the determination of the individual maximum exercise intensity on a treadmill (day 4, Fig 2) to exclude that symptoms did not develop because of ASA and alcohol, exercise-induced anaphylaxis, and cholinergic urticaria.[17]

Safety

Patients were under continuous inpatient monitoring by a medical student (D.K.) and a physician (K.B.) with emergency resuscitation equipment at hand.

Plasma gliadin concentrations

The serum concentration of gliadin was measured at timed intervals during challenge by using the method described by Matsuo et al.[18] Gliadin was extracted from 0.5 mL of serum with 1.5 mL of 70% ethanol. After vortexing for 1 minute, the mixture was centrifuged at 20,000*g* for 5 minutes at room temperature. The supernatant, after evaporation to dryness, was dissolved in kit sample dilution buffer, and the gliadin concentration was determined by using the Wheat Protein (Gliadin) ELISA Kit (Morinaga Institute of Biological Science, Yokohama, Japan), with native gliadin (Tokyo Kasei Kogyo, Tokyo, Japan) as the standard. The detection limit for this assay is 15 pg/mL.

SPTs

Patients underwent SPTs with proprietary wheat extract (Allergopharma, Reinbeck, Germany) and prick-to-prick tests with native wheat flour, gluten, and other cereal flours (results not shown). Results were positive if the mean wheal diameter was 3 mm or greater after 20 minutes. Histamine dihydrochloride (10 mg/mL; ALK-Abelló, Copenhagen, Denmark) and physiologic saline were used as positive and negative controls. Skin tests were repeated 30 minutes after 1000 mg of ASA.

Specific IgE

Total and specific IgE to wheat, gluten, and recombinant ω5-gliadin in serum were assayed with the ImmunoCAP/FEIA kit (Pharmacia, Uppsala, Sweden). Specific IgE levels of 0.35 kU_A/L or greater were defined as positive.

Gastrointestinal permeability

Gastrointestinal permeability was assessed by using the triple-sugar test.[19] The test measures urinary excretion of orally administered nonmetabolized sugar probes. Sucrose served as a marker for gastroduodenal permeability and the lactulose/mannitol ratio (permeability index) for small intestinal permeability. After overnight fasting and a pretest urine sample, subjects drank a solution containing 20 g of sucrose, 10 g of lactulose, and 5 g of mannitol dissolved in 100 mL of water. Urine was collected over 5 hours with hydrochloric acid as a preservative and stored at −20°C until analysis. Urine protein was removed with sulfosalicylic acid desalted with Amberlite MB-3 resin (Dow Chemicals, Midland, Mich). By using mesoerythritol and turanose as internal standards, the sugars were separated, analyzed, and quantified by means of HPLC with pulsed electrochemical detection (Dionex, Idstein, Germany).

Statistical analysis

Data are expressed as means ± SDs unless stated otherwise. Sensitivity, specificity, and positive and negative predictive values were calculated. Statistical analyses were performed by using the *t* test for paired values and the Wilcoxon *U* test. A *P* value of less than .05 was considered significant.

RESULTS

Gluten challenge

Eleven of 34 patients with a convincing history of WDEIA agreed to oral challenge tests with wheat products, cofactors, and exercise. The test result was positive in only 4 patients (36%; Fig 1). In the second part of the study, 16 patients consented to oral challenge with increasing amounts of gluten with or without cofactors. All had symptoms of urticaria (n = 15) or nausea and diarrhea (n = 1). In 5 patients we found associated weakness,