

In vitro demonstration of IgE antibody to folate-albumin in anaphylaxis from folic acid

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Background: Folic acid (the synthetic form of folate B vitamins in foods) is widely used in vitamin supplements. Anaphylaxis from ingestion or injection of folic acid suggests IgE antibody-mediated mechanisms, but this has not been demonstrated previously in vitro.

Objective: This study was conducted to better define the mechanism of folic acid hypersensitivity and cross-reactivity among folic acid congeners.

Methods: Skin testing was performed with folic acid congeners in a woman who developed anaphylaxis after ingestion of 2 different multivitamin preparations containing folic acid. In vitro immunologic serum studies were conducted using a folate-human serum albumin (HSA) conjugate prepared by a novel application of carbodiimide condensation.

Results: The patient had positive immediate-type skin test reactions to folic acid and several folate analogues including leucovorin (folinic acid). Urticaria developed during graded oral test dosing with leucovorin. Using a dot immunoblot assay or an ELISA for IgE antibody to folate-HSA, results of the patient's serum testing were positive, whereas results of sera from normal control subjects were negative, the first in vitro demonstration of IgE to a folic acid-protein conjugate. By ELISA, the positive result of the patient's serum was inhibited significantly by serum coincubation with folate-HSA, but not HSA or folic acid.

Conclusions: Immediate hypersensitivity to folic acid and possibly other vitamins can be mediated by IgE antibody to conjugates formed between vitamins and self-proteins or polypeptides. Leucovorin can have clinically important immunologic cross-reactivity with folic acid. A diet rich in natural folates (pteroylpolyglutamates) appears useful as a management strategy for providing adequate nutrition to patients with folic acid hypersensitivity. (*J Allergy Clin Immunol* 2000;106:386-9.)

Folic acid (pteroylmonoglutamic acid) is a 441-d B vitamin that is essential for DNA synthesis and other metabolic processes. Folic acid deficiency may lead to megaloblastic anemia, impaired growth, neural tube

Abbreviations used

EDAC: 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide

HSA: Human serum albumin

THFA: Tetrahydrofolic acid

defects, and other abnormalities.^{1,2} More recent evidence suggests that increased intake of folic acid is associated with decreased risk of atherosclerotic heart disease and certain malignancies.³⁻⁷ Accordingly, folic acid is increasingly used as a vitamin supplement, and now fortifies all enriched grain products in the United States to reduce the risk of neural tube defects in newborns.

Although there are a number of reports of anaphylaxis from ingestion of folic acid,⁸⁻¹⁰ to our knowledge IgE antibodies to folic acid have never been demonstrated in vitro. We report the first in vitro demonstration of IgE to folic acid in serum from a patient who developed anaphylaxis from vitamin supplements. Our results indicate that folic acid acts as a hapten in association with self-polypeptides or proteins.

CASE REPORT

A 32-year-old woman developed urticaria, facial angioedema, nausea, and repeated vomiting 20 minutes after ingesting a multivitamin tablet. The patient was treated in an emergency department and the reaction resolved over several hours. She had a similar reaction after ingestion of a different brand of multivitamin tablet several years earlier. She previously had tolerated a liquid high-potency vitamin mixture without adverse reaction. Constituents common to both vitamin tablets but absent in the liquid formula included folic acid, yellow dye No. 6, and numerous inert substances or trace minerals.

Immediate skin testing

Skin prick testing results were negative for reactivity to yellow dye No. 6, but markedly positive to folic acid 5 mg/mL (12-mm wheal/50-mm flare). Skin prick testing using serial 10-fold dilutions of 5 mg/stock solutions of folic acid and its congeners demonstrated the following positive endpoint titers: folic acid 1:10,000, tetrahydrofolic acid (THFA) 1:1000, methyltetrahydrofolic acid 1:100, and folinic acid (leucovorin) 1:1000. All these folic acid congeners have a hydroxyl group at position 4 of the pterin ring. However, the patient had a negative skin test result to methotrexate (5 mg/mL); methotrexate has an amino group at position 4 (Figure 1). Five normal control subjects had negative skin test results to the highest tested concentrations of folic acid, its congeners, and yellow dye No. 6.

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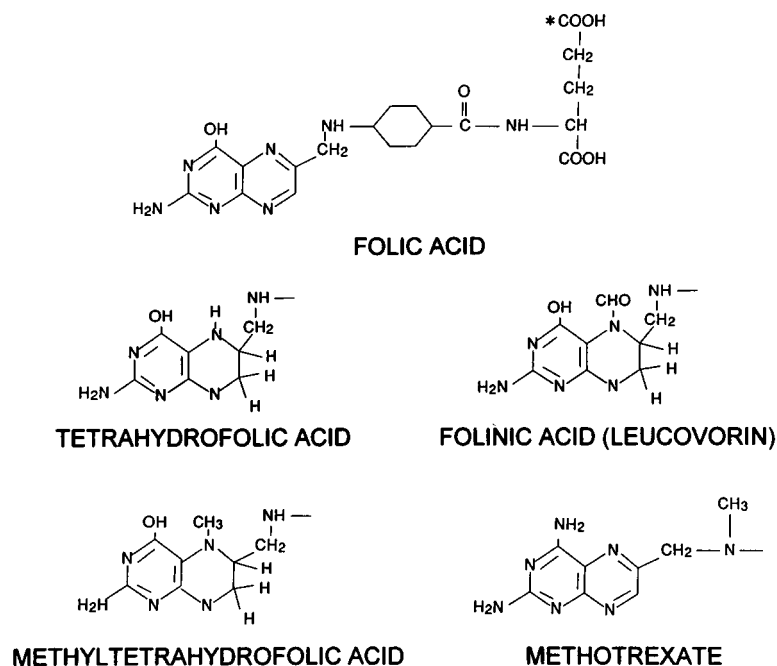


FIG 1. Structures of folic acid and its analogues. The *asterisk* indicates the site where polyglutamates are conjugated to folic acid in naturally occurring folates.

Graded challenge with folinic acid

For graded oral challenge with folinic acid (leucovorin), the patient sequentially received doses of 10 mg, 50 mg, and 100 mg with a 30-minute interval between each dose. Twenty minutes after the 100-mg dose, the patient developed generalized urticaria.

In vitro studies

Preparation of folate-human serum albumin conjugate. For in vitro antibody studies, a conjugate of folic acid and human serum albumin (HSA) was prepared by carbodiimide condensation, a technique that covalently links carboxyl groups of organic acids to free amino groups of proteins.¹¹ Folic acid was dissolved in a minimal volume of 0.1N sodium hydroxide before adding it to an HSA solution in 0.003 mol/L phosphate buffer, pH 6.3. While pH was maintained at 6.3, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, molecular weight 192) was added slowly. After stirring overnight in the dark, the solution was then extensively dialyzed (using a membrane with a molecular weight cutoff of 6000-8000) for 3 days with 0.005 mol/L PBS at pH 7.2 to remove free folic acid and EDAC. The resulting solution of the folate-HSA conjugate retained the characteristic yellow color of folic acid. By gel electrophoresis, migration of the folate-HSA conjugate was altered from that of HSA. The protein concentration of the conjugate was assayed with BCA Protein Assay (Pierce, Rockford, Ill).

Dot immunoblot assay for IgE to folate-HSA. A dot immunoblot assay for IgE to folic acid-HSA was performed by applying drop aliquots of folic acid-HSA solution to nitrocellulose. After air drying, the following reagents were added with appropriate dilutions, washes, and incubation periods: 1% fetal calf serum in Tris-buffered saline solution, human serum, rabbit anti-human IgE, goat anti-rabbit gamma globulin-alkaline phosphatase conjugate, and finally

nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate substrate (BioRad, Hercules, Calif).

ELISA for IgE to folate-HSA and inhibition studies. Briefly, the following reagents were added to microtiter plates using appropriate dilutions, washes, and incubation periods: folate-HSA, goat anti-human IgE, rabbit anti-goat gamma globulin-alkaline phosphatase conjugate, and p-nitrophenol phosphate substrate. The optical densities of results were read at 405 nm. For inhibition studies, dilutions of folate-HSA, HSA, or free folic acid were coincubated with serum for 30 minutes at 37°C.

RESULTS

Dot immunoblot assay for IgE to folate-HSA

The results of the dot immunoblot assay for IgE to folate-HSA are shown in Figure 2. Serum from the folic acid-sensitive patient yielded positive findings, whereas test results on sera from 11 normal control subjects were negative.

ELISA for IgE to folate-HSA and inhibition studies

In the ELISA for IgE to folate-HSA, 1:10 serum from the folic acid-sensitive patient had an optical density that was at least twice that from 11 samples of control sera that yielded negative results. Figure 3 shows the results of ELISA inhibition studies with folate-HSA, HSA, and unconjugated folic acid at a 1:10 dilution of serum from the folic acid-sensitive patient. Serum coincubation with folate-HSA caused significant inhibition of the ELISA, whereas coincubation with HSA caused no inhibition, and unconjugated folic acid caused minimal inhibition.



FIG 2. Results of the dot immunoblot assay for IgE to folate-HSA. 1:10 Serum (1:10) from the folic acid-sensitive patient (position 1) yielded positive findings, whereas results of serum testing from 11 normal control subjects (positions 2 through 12) were negative. Folate-HSA control positions without addition of human serum were probed with the rabbit- and goat-conjugated secondary antibodies (position 13) or goat-conjugated secondary antibody (position 14).

ELISA Inhibition Studies

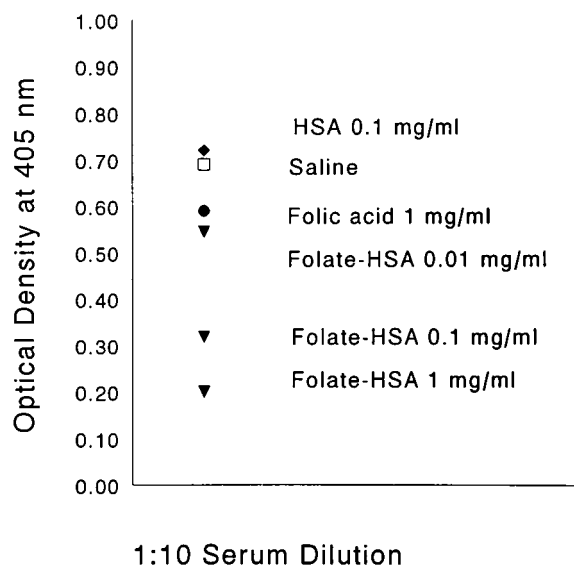


FIG 3. Results of ELISA inhibition studies with folate-HSA and HSA at a 1:10 dilution of serum from the folic acid-sensitive patient. Compared with the result without inhibitors, serum coin-cubation with folate-HSA caused significant inhibition of the ELISA, whereas HSA coin-cubation did not, and unconjugated folic acid caused relatively minimal inhibition.

DISCUSSION

To our knowledge, this is the first in vitro demonstration of IgE to folic acid. Our in vitro studies and the positive immediate-type skin test results to folic acid confirm that our patient's anaphylaxis was an IgE-mediated reaction to folic acid. Because serum coin-cubation with free folic acid caused only minimal inhibition of our ELISA for IgE to folate-HSA, whereas conjugated folate-HSA caused significant inhibition, our in vitro assays also suggest that folic acid can—and possibly must—act as a hapten in immunologic reactions. This is not surprising considering that the molecular weight of folic acid, 441 d, is well below the 1000-d threshold usually required for an agent to be recognized as a complete antigen or allergen.¹²

The ability of folic acid solutions to elicit positive immediate skin test reactions suggests that folic acid must be capable of rapidly combining with self-proteins or polypeptides in the skin to form a complete allergen. Although our

in vitro assays for IgE antibody used a folic acid-HSA conjugate prepared by chemical conjugation, in vivo processes likely permit folic acid or its metabolites to act as a hapten associated with a number of polypeptides or proteins. Folic acid (pteroylglutamic acid) is the unconjugated, synthetic form of naturally occurring folate vitamins (pteroylpolyglutamates) that are composed of folic acid and polyglutamate moieties (5 to 8 glutamate residues) conjugated at the gamma carbon positions (Figure 1). During digestion, the polyglutamate moieties are cleaved in the gastrointestinal lumen before absorption of the reduced form of folic acid, THFA, which is frequently methylated.¹³ There are several mechanisms by which folic acid or THFA could then serve as a hapten: (1) covalent conjugation to polyglutamate moieties,¹ (2) reversible binding to soluble and membrane-bound human folate-binding proteins¹⁴⁻¹⁶ or to bovine folate-binding protein absorbed from ingested dairy products, and (3) physical association with enzymatic proteins that are dependent on THFA as a coenzyme.¹

Our patient and others reported to have anaphylaxis from folic acid vitamin supplements apparently have been able to tolerate ingestion of most foods containing natural folates, but it may be possible for patients to have reactions from eating foods, particularly grain products enriched with folic acid.

On the basis of skin testing, ELISA inhibition studies, and a positive oral challenge to leucovorin (folic acid), our patient had cross-reactivity with several folic acid congeners. Specifically, our patient had positive skin test results to all agents that have a hydroxyl group at position 4 of the pterin ring. However, she had a negative skin test reaction to methotrexate, which has an amine group at position 4. This pattern of cross-reactivity is similar to skin test results in one of the patients with folic acid hypersensitivity described by Woodliff and Davis.¹⁰ However, a patient described by Chanarin and Fenton⁹ had positive skin test results to folic acid and methotrexate, but not to other analogues such as THFA, indicating that the epitopes recognized by antibodies to folate determinants can vary, as can the pattern of cross-reactivity with different folate analogues. The development of generalized urticaria with folinic acid (leucovorin) in our patient with folic acid sensitivity is apparently the first report of clinically significant cross-reactivity between folic acid and leucovorin. Patients with folic acid sensitivity therefore should be considered at potentially increased risk of sensitivity to leucovorin, and any patients with immediate-type reactions to leucovorin may be at increased risk of reactions to folic acid vitamin supplements.

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