

Anaphylaxis to riboflavin (vitamin B₂)

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Background: Vitamin supplements are used more commonly in normal healthy subjects than in patients with vitamin deficiency. Thiamine (vitamin B₁) is the vitamin that most frequently induces allergic reactions. To the best of our knowledge, no case of anaphylaxis to riboflavin (vitamin B₂) has thus far been reported in the literature.

Objective: We describe a previously healthy 15-year-old boy in whom anaphylaxis developed several times after he drank one soft drink or took a single multivitamin tablet. This study was done to determine which of the many components found in the soft drink and vitamin tablet caused the anaphylactic reaction.

Methods: In an outpatient clinic with the availability of complete resuscitative procedures, we performed single-blind prick skin tests and intradermal skin tests on the patient with various pure vitamin components of the soft drink and the multivitamin tablet. Physiologic saline and histamine were used for negative and positive controls, respectively.

Results: Riboflavin, a component of both the soft drink and the vitamin tablet, produced positive reactions on intradermal skin tests in the patient. Positive reactions were not present in the normal control subjects.

Conclusions: Riboflavin is a previously unreported cause of anaphylaxis. Free-form riboflavin may potentially be associated with an anaphylactic reaction. It is a vitamin widely used in many patients with chronic disease and in healthy subjects. Vitamin B₂ must be considered as a cause of anaphylaxis.

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INTRODUCTION

Anaphylaxis is a severe, life-threatening, systemic allergic reaction and is considered a medical emergency. Most frequently, the initial manifestations of anaphylaxis are respiratory, cardiovascular, cutaneous, and gastrointestinal symptoms; however, fulminant cardiovascular collapse may be the only manifestation.

Vitamin supplements are used more commonly in normal healthy subjects than in patients with vitamin deficiency. Thiamine (vitamin B₁) is the vitamin that most frequently induces allergic reactions¹ and sometime causes anaphylactic shock.^{2–7} Ribofla-

vin (vitamin B₂) has not previously been documented as being associated with the development of urticaria, angioedema, or anaphylaxis. We describe a patient in whom an anaphylactic reaction developed after he took one multivitamin tablet, which contained multivitamins and trace elements. Vitamin B₂ was found to be the allergic component that induced this anaphylactic shock condition, confirmed by single-blind prick and intradermal skin tests. In this report, we present the first case of anaphylaxis attributable to vitamin B₂. Accordingly, vitamin B₂ must now be considered as a cause of anaphylaxis.

CASE REPORT

After a 15-year-old boy took one multivitamin tablet, flushing and a general papular rash developed, followed by dizziness, dyspnea, nausea, and severe angioedema of the face 40 minutes later. The patient was drowsy in the emergency department, and hypotension plus tachycardia subsequently oc-

curred (blood pressure, 67/48 mm Hg; pulse rate, 131 beats/minute; respiratory rate, 28 breaths/minute; body temperature, 36° C). He was treated with subcutaneously administered epinephrine and physiologic saline infusion. Diphenhydramine and hydrocortisone injections were given. His vital signs became more stable approximately 30 minutes later (blood pressure, 118/74 mm Hg; pulse rate, 78 beats/minute), and he recovered consciousness shortly thereafter.

The patient had no history of atopic diseases, and he denied having any previous adverse drug reactions. He reported, however, that he had experienced dizziness many times after drinking one particular brand of soft drink with yellow color, and he had lost consciousness twice after drinking that soft drink approximately 5 to 6 years previously. Each episode necessitated a visit to the emergency department and the subcutaneous administration of epinephrine. The patient had not drunk the yellow soft drink since then. He had no family history of angioedema or sudden death. He was a nonsmoker and took no regular medications.

Physical examination was unremarkable. The hemoglobin concentration, leukocyte counts, platelet count, total immunoglobulin (Ig)E, absolute eosinophil count, and C3 serum level were normal. The C4 serum level (14.5 mg/dL; normal range, 15 to 45) was mildly decreased. Results of an allergy panel consisting of common allergens and fluoroimmunoassay for specific IgE were all negative.

The multivitamin tablet that the patient took contained vitamins B₁ (100 mg of thiamine hydrochloride), B₂ (100 mg of riboflavin), B₆ (100 mg of pyridoxine hydrochloride), B₁₂ (500 µg of cyanocobalamin concentrate), and vitamin C (500 mg of ascorbic acid). We found that the soft drink had

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vitamin additives, including vitamin B₁ (0.6 ppm of thiamine hydrochloride), vitamin B₂ (8.9 ppm of riboflavin), and vitamin C (20 mg/dL of ascorbic acid). On the basis that the episodes of anaphylaxis had a strong temporal relationship to the ingestion of the soft drink and the multivitamin tablet, we considered that one of the vitamins may have caused the anaphylactic reaction.

After providing informed consent, the patient underwent a series of outpatient clinic-based, single-blind, placebo-controlled prick and intradermal skin tests. All the aforementioned compounds were tested on day 20: vitamin B₁ (thiamine hydrochloride), 1 mg/mL and 10 mg/mL; vitamin B₂ (riboflavin 5'-phosphate sodium), 0.1 mg/mL and 1 mg/mL; vitamin C (ascorbic acid), 10 mg/mL and 50 mg/mL; positive control (histamine), 1 mg/mL; and negative control (placebo), physiologic saline. All the test samples were provided by China Chemical & Pharmaceutical Company, Taipei, Taiwan, and all the test samples fulfilled the requirements of the United States Pharmacopeia.^{8,9}

Concentrations used for skin testing had previously been established in a group of 10 control subjects with good tolerance of these agents. The patient was restricted from taking any antihistamine drug for 7 days before the skin tests. The prick tests showed only local weakly erythematous change (wheal, 0 mm; erythema, 4 mm) with riboflavin (1 mg/mL). The intradermal skin test result was considered positive if the wheal was more than 5 × 5 mm and had an accompanying flare >6 mm at

15 minutes. The reagents, doses, and their local reactions are outlined in Table 1. Only histamine and vitamin B₂ induced a positive response in the patient. Riboflavin resulted in a 20-mm wheal at a concentration of 1 mg/mL and a 12-mm wheal at a concentration of 0.1 mg/mL. Complete resuscitative measures were available. Baseline spirometry values (forced expiratory volume in 1 second and forced vital capacity) and blood pressure were measured before the skin tests, and repeated measurements were done during and after administration of the reagents. Subjective symptoms were also recorded. The patient experienced severe itching and mild tenderness around the injection sites of the vitamin B₂ and the histamine. No change was noted in the blood pressure or the results of spirometry.

The next day, the patient's serum histamine value was normal. We also performed an in vitro histamine release assay for the patient and five "negative" control subjects. All participants had a significant polymorphonuclear cell histamine release after riboflavin stimulation at a 2.5 mg/mL concentration, which continued to a concentration of 0.0005 mg/mL. No significant difference was noted in polymorphonuclear cell histamine release between the patient and the negative control subjects. Vitamin B₁ and vitamin C induced no significant increase in histamine release in comparison to the spontaneous histamine release. At concentrations that exceeded 50 mg/mL of the compounds used, a nonspecific tenderness to histamine release was

observed in the negative control subjects as well as the study patient.

The patient still had a strongly positive response to the vitamin B₂ intradermal skin test on day 50 (Table 2). All compounds with negative results by intradermal skin testing were subsequently given to the patient either orally or intravenously. Administration of vitamins B₁, B₆, B₁₂, and C caused no symptoms. The patient refused to undergo challenge tests with orally or parenterally administered vitamin B₂.

On the basis of the skin test results, the patient was advised to exclude free-form riboflavin-containing products from his diet. He was also advised to carry epinephrine for self-administration and was instructed in its use. During the 13 months since then, he has had no adverse allergic reactions.

DISCUSSION

To our knowledge, anaphylaxis from riboflavin has not heretofore been described. A computer-based search of the MEDLINE, Current Contents, and EMBASE databases showed no previously reported cases of urticaria or anaphylaxis attributable to vitamin B₂. Vitamin B₂ supplements are common in daily life in the form of multivitamin tablets and in several types of soft drinks. This report is the first published case of anaphylaxis attributable to vitamin B₂ confirmed with intradermal skin testing. Skin tests have been valuable in the diagnosis of anaphylactic sensitivity to a variety of antigens. In our study, the presence of a positive response to intradermal skin testing suggested an immediate histamine release induced by vitamin B₂. Because administration of the same concentration yielded negative results in control subjects, the response was not an irritative reaction. Intradermal skin tests are more sensitive than prick skin tests. If skin prick tests are negative, intradermal skin tests must be performed.

We postulate that, for this patient, the reactions observed may have been a direct effect of vitamin B₂ leading to both vasodilatation and bronchoconstriction in conjunction with the triggering of mast cell degranulation. The

Table 1. Results of Intradermal Skin Tests on Day 20

Test sample	Concentration (mg/mL)	Wheal (mm)	Erythematous change (mm)
Thiamine	10	0	0
Thiamine	1	0	0
Riboflavin	1	20	50
Riboflavin	0.1	12	38
Ascorbic acid	50	0	0
Ascorbic acid	10	0	0
Positive control (histamine)	1	15	47
Negative control (physiologic saline)	0.9%	0	0

Table 2. Results of Intradermal Skin Tests on Day 50

Test sample	Concentration (mg/mL)	Wheal (mm)	Erythematous change (mm)
Thiamine	10	0	0
Riboflavin	1	13	57
Riboflavin	0.1	13	52
Riboflavin	0.01	8	42
Riboflavin	0.001	2	11
Ascorbic acid	10	0	0
Positive control (histamine)	1	16	55
Negative control (physiologic saline)	0.9%	0	0

serum histamine test may detect mast cell mediator release in both anaphylaxis and mastocytosis.^{10,11} In this case, a serum specimen was not obtained immediately after the anaphylactic reaction in the patient; the patient's serum histamine value was normal.

The *in vitro* histamine release indicated that the patient's polymorphonuclear cells were able to release histamine after stimulation with vitamin B₂. The same reaction, however, was also noted in negative control subjects. We found that vitamin B₂ is a strong reagent for *in vitro* polymorphonuclear cell histamine release.

Riboflavin is a water-soluble vitamin. Vitamin B₂ is essential for the normal release of energy from carbohydrate, protein, and fat in food. Further, vitamin B₂ is important for normal growth and development, the production of and regulation of certain hormones, and the formation of erythrocytes. Numerous food sources are rich in vitamin B₂, including milk and related products, eggs, grain products, lean meat, liver, poultry, fish, and dark green vegetables. Our patient had never experienced an adverse reaction to consumption of these common foods. Although the reason is unclear, this outcome might be attributable to the conjugation of riboflavin with other components in natural food.

In plant and animal tissues, riboflavin links with phosphoric acid as flavin mononucleotide or riboflavin 5'-phosphate, which, together with adenosine monophosphate, forms flavin adenine dinucleotide.¹² The human myeloma protein, IgG^{GAR}, was discovered, iso-

lated, and characterized as riboflavin binding by Farhangi and Osserman.¹³ Chang et al^{14,15} found that the main feature of riboflavin binding is a very strong antibody-hapten interaction. Flavin adenine dinucleotide binds to IgG^{GAR}, with a 15-fold decrease in its binding constant relative to riboflavin, and flavin mononucleotide also has a 5-fold decreased affinity relative to riboflavin.¹⁶ Therefore, the free-form vitamin B₂ in the soft drink and the vitamin tablet consumed by our patient could be a stronger hapten for the induction of an anaphylactic reaction than conjugated riboflavin in natural food. Perhaps IgE-specific antibodies to riboflavin-conjugated protein may be found in this patient.

CONCLUSION

Vitamin supplements are used more commonly in normal subjects than in patients with vitamin deficiency. Thiamine (vitamin B₁) is the vitamin that most frequently induces allergic reactions, including anaphylactic shock. In addition, vitamin A, vitamin C, and solutions of multivitamins may induce anaphylactic reactions.¹⁷⁻¹⁹ In this study, we present the first published case of anaphylaxis to riboflavin (vitamin B₂). Therefore, vitamin B₂ must be considered as a cause of anaphylaxis.

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