

Food and drug reactions and anaphylaxis

IgE-mediated allergy and desensitization to factor IX in hemophilia B

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Background: We describe two patients with factor IX deficiency and high levels of inhibitors to factor IX who developed anaphylaxis to factor IX.

Objective: The aim of this study was to develop a skin test, RAST, and desensitization protocol for factor IX allergy.

Methods: The patients were evaluated by skin test and RAST to factor IX. They also underwent desensitization to factor IX. **Results:** Both patients had positive skin test and RAST reactions to factor IX. Control subjects had negative reactions. Both patients were successfully desensitized to factor IX by using two different desensitization protocols. The patients' skin test and RAST reactions to factor IX converted to negative after desensitization.

Conclusions: IgE-mediated reactions to factor IX do occur and may be diagnosed with the use of skin test and RAST. Patients with this type of reaction may be successfully desensitized to factor IX. (*J Allergy Clin Immunol* 1998;102:113-7.)

Key words: Allergy, anaphylaxis, desensitization, factor IX deficiency

Hemophilia B is an X-linked disorder in which there is a congenital deficiency of coagulation factor IX clotting activity. Patients with hemophilia B are treated by intravenous replacement of factor IX, and a number of highly purified factor IX concentrates are available for this purpose. A serious complication of repeated factor IX infusions in a subset of these patients is the development of anaphylactic reactions associated with the infusions.¹⁻⁴ Anaphylactic reactions to factor IX are more prevalent in patients with severe hemophilia B (< 1% factor IX) who are also predisposed to developing inhibitors to factor IX. Inhibitors are neutralizing antibodies to factor IX of the IgG isotype that are predominantly of IgG4 and IgG1 subclasses.⁵⁻⁸ These inhibitors prevent the achievement of hemostatic levels of factor IX in reaction to infusion of factor IX concentrates. Sawamoto et al.⁴ measured factor IX-specific IgG subclasses in six patients with factor IX deficiency and inhibitors to factor IX who had anaphylaxis to factor IX. They found an increase in factor IX-specific IgG1. To our knowledge the role of

Abbreviations used

HSA: Human serum albumin

PCC: Prothrombin complex concentrate

IgE-mediated allergy in the anaphylactic type of reactions to factor IX infusions has not been clearly demonstrated.

In patients with high levels of inhibitors, bleeding episodes may be controlled with activated prothrombin complex concentrates (PCCs), but these products also contain variable quantities of factor IX. Anaphylaxis to all factor IX-containing products further complicates the treatment course of patients with hemophilia B with inhibitors, and therefore desensitization to factor IX is crucial in the optimal management of such patients. To our knowledge, desensitization to factor IX has not been previously described.

We report two patients with severe factor IX deficiency and high levels of factor IX inhibitors who developed anaphylaxis to factor IX. Both patients had positive RAST and skin test reactions to factor IX and were successfully desensitized to it. In both patients levels of circulating IgE antibodies to factor IX significantly decreased after the desensitization process.

CASE HISTORIES

Patient 1

A 9-year-old boy with severe factor IX deficiency and a high titer inhibitor to factor IX (maximum, 51 Bethesda units) had been treated with FEIBA (Immuno AG, Vienna, Austria), an activated PCC, for joint hemarthroses over several years. The patient had a mild rash during an infusion at 2 years of age after which he was started on oral diphenhydramine pretreatment before his infusions. Except for this episode, the infusions were well tolerated until 7 years of age. At this time the patient developed generalized urticaria, angioedema of the lips and eyelids, cough, abdominal discomfort, and tachycardia within 10 minutes of receiving an intravenous infusion of FEIBA. His symptoms resolved with intravenous infusion of diphenhydramine and dexamethasone. Intradermal skin test reactions to FEIBA and three factor IX preparations were positive at the 1:10 dilution. RAST reactions to Mononine (Armour Pharmaceutical, Kankakee, Ill.), a monoclonal antibody-purified factor IX product, were positive.

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Subsequently, the patient underwent desensitization to factor IX. The desensitization protocol was based on the successful factor VIII desensitization protocol by Jamieson et al.,⁹ with minor modifications. However, as higher doses of factor IX were administered, the patient experienced generalized urticaria that responded partially to intravenous methylprednisolone and diphenhydramine and required drastic slowing of the infusion rate. Eventually, the patient was able to tolerate factor IX administration at near-normal infusion rates. To maintain the desensitized state, the patient has continued to receive maintenance treatment with factor IX, and with this regimen he has been able to tolerate FEIBA infusions for treatment of hemarthroses without reactions. His skin test reactions to factor IX and FEIBA, performed 3 months after desensitization, and his RAST reaction to factor IX, performed 5 months after desensitization while he was receiving daily maintenance treatment with Factor IX, were negative.

Patient 2

This patient, who is the first cousin of patient 1, is a 2-year-old boy with severe factor IX deficiency and a high titer inhibitor to factor IX (maximum, 67.5 Bethesda units). He was treated with factor IX concentrate (100 U/kg) every other day for chronic subdural hemorrhages. The infusions were well tolerated until 10 months of age. At this time the patient had a generalized maculopapular rash during an infusion with factor IX. The rash persisted throughout the remaining 2 days of factor IX infusion and resolved within a few hours of discontinuing factor IX. At 11 months of age, he had generalized urticaria, angioedema, projectile vomiting, and wheezing within 5 minutes of an intravenous push with FEIBA. The anaphylaxis was treated with three injections of epinephrine, methyl prednisolone, and diphenhydramine, and the patient was admitted to the intensive care unit for 1 day. Intradermal skin test reactions to three different factor IX products were positive at the 1:10 dilution (Table I). RAST reaction to factor IX was positive (Table II).

At 14 months of age the patient underwent successful desensitization. On the basis of the experience with his cousin, a desensitization protocol was constructed in which the dose escalation was more gradual, and continuous infusion over several hours was used for higher doses. This new protocol (Table III) was well tolerated without any reactions. Similarly to patient 1, patient 2 has continued to receive maintenance infusions with factor IX to maintain the desensitized state. His skin test and RAST reactions to factor IX, performed 2 months after desensitization, converted to negative, and he has been able to tolerate factor IX and activated PCC infusions without any allergic reactions.

METHODS

Skin tests to factor IX

Skin prick tests to two different highly purified factor IX concentrates (Mononine and Alphanine SD [Alpha Therapeutic Corpo-

ration, Los Angeles, Calif.]) and two activated PCCs (FEIBA and Konyne 80 [Miles Inc., Elkhart, Ind.]) were performed by using a Multi-Test device (Center Laboratories, Port Washington, N.Y.). The full-strength concentration of 25 U/ml was used for each product on the basis of the concentration used for factor VIII skin testing by Jamieson et al.⁹ Intradermal skin tests were performed with 1:100 and 1:10 concentrations of each product with normal saline as both diluent and negative control. The reactions were assessed at 15 minutes, and a wheal of greater than 8 mm with a flare of at least 10 mm was interpreted as a positive test reaction as long as the wheal was at least 3 mm larger than that caused by the negative saline control. The mothers of patient 1 and patient 2 served as control subjects and gave informed consent before testing. Because of the possible risks involved in exposure to blood products, additional control subjects were not tested.

RASTs to factor IX

Mononine, mouse IgG (Sigma Chemical co., Saint Louis, Mo.), and human serum albumin (HSA) were each coupled to CNBr-activated Sepharose 4B beads (Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's protocol. Mononine was the factor IX product used for the test because it was the product selected for the desensitization protocols. It is a purified factor IX concentrate prepared from pooled human plasma by using a murine monoclonal antibody to factor IX, and it contains a small amount of mouse protein. For this reason, mouse IgG-Sepharose beads were used as controls. HSA bound to Sepharose was used as negative control.

Sera from the two patients and from the control subjects were analyzed according to the method of Hamilton and Adkinson,¹⁰ with some modifications. Briefly, allergen-coated Sepharose beads were prepared as a 1% suspension in RAST buffer (Phadebas RAST kit; Pharmacia, Uppsala, Sweden). Five hundred microliters of the suspension was incubated with 100 microliters of serum diluted 1:2 in RAST buffer. The samples were rotated overnight at room temperature. On day 2, the beads were washed three times with RAST buffer and then incubated with 200 microliters of ¹²⁵I-labeled anti-human IgE (Phadebas RAST, Pharmacia) overnight at room temperature with orbital rotation. After four washes with RAST buffer, the radioactivity bound to the beads was measured as counts per minute by using a gamma scintillation counter (LKB). On the basis of a common method for RAST interpretation,¹⁰ a RAST ratio was defined as the ratio of the counts per minute obtained with the patient's serum to the negative control serum. Patient/negative serum ratios greater than 2.5 were considered positive. All tests were done in triplicates.

By using a similar method, IgE anti-IgG was measured in both predesensitization sera by measuring bound radioactivity to IgG Sepharose 6 Fast Flow (Pharmacia Biotech) beads.

Desensitization to factor IX

A different desensitization protocol was designed for each of the two patients. The first protocol was based on a published factor VIII desensitization protocol by Jamieson et al.,⁹ with minor modifications. Briefly, intravenous infusions of factor IX were started at 0.01 U/kg, and doses were doubled at 10-minute intervals to reach a cumulative dose of 100 U/kg. The second protocol used in patient 2 was based on the previous experience in patient 1. It consisted of more gradual increments in dosage and an increase in the infusion intervals and duration as higher doses of factor IX were administered. Once the cumulative dose of 100 U/kg was reached, the infusion rate was gradually increased (Table III).

The protocols for evaluation and treatment of both subjects were approved by the Children's Hospital Committee on Clinical Investigation.

TABLE I. Results of factor IX skin testing

	Factor IX Mononine (1:10 ID)	Factor IX Alphanine SD (1:10 ID)	APCC FEIBA (1:10 ID)	APCC Konyne 80 (1:10 ID)	Histamine (prick)	Saline control (1:10 ID)
Patient 1	1+	2+	1+	1+	2+	0
Control subject 1	0	0	0	0	1+	0
Patient 2	2+	1+	ND	2+	2+	0
Control subject 2	0	0	ND	0	2+	0

Skin prick test reactions to full strength and intradermal test reactions to 1:100 dilution of all products were negative in both patients and control subjects. Skin test results: 0, negative; 1+, wheal of 5 to 10 mm with flare of 10 to 20 mm; 2+, wheal of 5 to 10 mm with flare of 21 to 30 mm.¹⁷
APCC, Activated prothrombin complex concentrate; 1:10 ID, intradermal testing with 1:10 dilution; ND, not done.

TABLE II. Results of RASTs

	Patient serum (cpm)			Control serum (cpm)			RAST ratio			Mean RAST ratio ($\pm$ SEM)
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	
Factor IX–Sepharose										
Patient 1	305	372	734	76	47	190	4	8	3.9	5.3 $\pm$ 1.1
Patient 2	1698	1719	4227	117	135	418	14.5	12.7	10.1	12.4 $\pm$ 1.8
Patient 1 (after D)	179	1247	349	73	574	300	2.4	2.2	1.2	1.9 $\pm$ 0.3
Patient 2 (after D)	160	157	145	117	112	111	1.4	1.4	1.3	1.4 $\pm$ 0.03
HSA–Sepharose										
Patient 1	139	274	234	134	364	264	1	1	0.9	1.0 $\pm$ 0.06
Patient 2	180	417	384	133	312	432	1.4	1.3	0.9	1.2 $\pm$ 0.1
Mouse–Sepharose										
Patient 1	1665	1390	829	1477	1339	962	1.1	1	0.86	1.0 $\pm$ 0.06
Patient 2	2645	2015	1204	1758	1402	1104	1.5	1.4	1.1	1.3 $\pm$ 0.1

Post-desensitization samples from patients were obtained 5 months and 2 months after the desensitization process in patients 1 and 2, respectively. Both patients were still receiving daily factor IX infusions when the postdesensitization samples were obtained.
Exp, Experiment; D, desensitization.

RESULTS

Skin tests to factor IX

Skin prick test reactions at full strength concentration and intradermal test reactions at 1:100 dilution for all factor IX concentrates were negative in both patients and control subjects (data not shown). Both patients had positive reactions to both purified factor IX products (i.e., Mononine and Alphanine SD) injected intradermally at the 1:10 dilution (Table I). The control subjects had no reaction to the same products. Patient 1 was also skin tested with two activated PCCs, FEIBA and Konyne 80. Patient 1, but not the control subject, had a positive reaction to both activated PCCs at the 1:10 concentration (Table I).

RAST to factor IX

RAST reactions to factor IX were significantly positive in both patients; the RAST ratio was 5.3 ± 1.1 for patient 1 and 12.4 ± 1.8 for patient 2 (Table II). Neither patient had evidence of nonspecific IgE binding to HSA or mouse IgG (Table II). IgE anti-IgG measured in pre-desensitization sera was negative in both patients (data not shown).

Desensitization to factor IX

Desensitization to factor IX was successful in both patients. The more rapid desensitization protocol in

patient 1 was complicated by persistent severe urticaria that first appeared at the cumulative dose of 65 U/kg and only partially responded to methyl prednisolone and diphenhydramine. After a few unsuccessful attempts to resume the rapid desensitization at a lower dosage, the infusion rate was slowed over 24 hours, and the dosage was gradually increased. By the end of day 8, the patient was able to tolerate a full dose of factor IX over 20 hours, and the infusion rate was gradually increased over the next 5 days. The patient continued to receive systemic corticosteroids and diphenhydramine throughout the desensitization process.

The more gradual desensitization protocol used for patient 2 (Table III) was designed on the basis of the experience with patient 1. It was completely uneventful, with no need for systemic steroids or antihistamines, and was accomplished over an overall shorter period of time.

After desensitization, both patients were able to tolerate infusions of factor IX and FEIBA without any reactions. Postdesensitization skin test and RAST reactions to Mononine, performed while the patients were still receiving daily infusions with factor IX, converted to negative. To maintain the desensitized state, both patients continued to receive daily factor IX infusions. The frequency of the infusions was later decreased to every other day on the basis of the half-life of factor IX, which is about 25 hours. This every other day infusion regimen

TABLE III. Factor IX desensitization protocol for patient 2

	Dose (U/kg)	Cumulative dose (U/kg)	Method	Interval from infusion of previous dose	Reactions
Day 1	0.01	0.01	Slow IV push	0 minutes	None
	0.02	0.03	Slow IV push	10 minutes	None
	0.04	0.07	Slow IV push	10 minutes	None
	0.08	0.15	Slow IV push	10 minutes	None
	0.10	0.25	Slow IV push	10 minutes	None
	0.2	0.45	Slow IV push	20 minutes	None
	0.4	0.85	Slow IV push	20 minutes	None
	0.8	1.65	Slow IV push	20 minutes	None
	1.5	3.15	Slow IV push	20 minutes	None
	3.0	6.15	Continuous infusion over 30 minutes	—	None
	6.0	12.15	Continuous infusion over 30 minutes	—	None
	8.0	20.15	Continuous infusion over 30 minutes	—	None
	9.0	29.15	Continuous infusion over 60 minutes	—	None
	11.0	40.15	Continuous infusion over 60 minutes	—	None
	12.0	52.15	Continuous infusion over 60 minutes	—	None
	14.0	66.15	Continuous infusion over 60 minutes	—	None
	16.0	82.15	Continuous infusion over 60 minutes	—	None
	18.0	100.15	Continuous infusion over 60 minutes	—	None
Day 2	100	100	Continuous infusion over 10 hours	—	None
Day 3	100	100	Continuous infusion over 8 hours	—	None
Day 4	100	100	Continuous infusion over 6 hours	—	None
Day 5	100	100	Continuous infusion over 4 hours	—	None
Day 6	100	100	Continuous infusion over 2 hours	—	None
Day 7	100	100	Continuous infusion over 1 hour	—	None
Day 8	100	100	Continuous infusion over 30 minutes	—	None

has been adequate in maintaining the desensitized state in both patients.

DISCUSSION

We report two patients with severe factor IX deficiency and inhibitors to factor IX who developed anaphylaxis to factor IX-containing concentrates. Skin test and RAST reactions to factor IX were positive in both patients. Both patients were successfully desensitized to factor IX; however, the more rapid desensitization protocol used for patient 1 was complicated by persistent urticaria. We therefore recommend the protocol used for the desensitization of patient 2 (Table III). Skin test and RAST reactions to factor IX converted to negative after desensitization, and both patients were able to tolerate factor IX infusions without any reactions.

The diagnosis of IgE-mediated allergy to factor IX in these two patients is supported by both clinical and laboratory data. They both had typical anaphylaxis within 5 to 10 minutes of infusion with factor IX. Both patients had positive skin test reactions to multiple factor IX preparations and demonstrated a positive RAST reaction to factor IX. The positive RAST reactions are unlikely to be nonspecific or caused by contaminants because neither one of the patients had a positive reaction to HSA or mouse IgG. This is important because the factor IX product, Mononine, that was used in the RAST is purified by using murine monoclonal antibody to factor IX.

Successful desensitization to factor IX in both patients enabled them to receive factor IX and activated factor IX complex products after desensitization without any reactions. The more rapid desensitization protocol used for patient 1 was complicated by persistent urticaria, which did not improve with intravenous methyl prednisolone and diphenhydramine and resolved only after drastic slowing of the infusion rate, dosage, and intervals. The more gradual desensitization protocol used for patient 2 was uneventful. A similar situation has been observed in patients with insulin allergy who can have urticaria during rapid desensitization with insulin and are more likely to tolerate a more gradual desensitization over several days.¹¹⁻¹⁵ Interestingly, many patients with insulin allergy will also have IgG-blocking antibodies associated with insulin resistance similar to the IgG factor IX inhibitors.

Sawamoto et al.⁴ measured factor IX-specific IgG subclasses in six patients with factor IX deficiency and inhibitors to factor IX who had anaphylaxis to factor IX and found an increase in factor IX-specific IgG1. Because factor IX inhibitors are known to be predominantly of the IgG4 and IgG1 subclasses,⁸ the clinical relevance of this finding is not clear. Both of our patients continued to demonstrate high levels of factor IX inhibitors after desensitization but were able to tolerate factor IX infusions without severe immediate-type allergic reactions. Their factor IX-specific IgE was significantly reduced after successful desensitization. This sug-

gests that our patients' anaphylactic reactions were IgE-mediated, whereas a second class of antibody, presumably IgG1, was responsible for the neutralization of factor IX. It is possible, however, that competition from factor IX-specific IgG may have spuriously lowered our patients' RAST reactions.

Anaphylactic reactions to factor IX-containing concentrates occur predominantly in patients with undetectable levels of factor IX caused by major disruptions in the factor IX gene.³ Patients with hemophilia B who have severe factor IX deficiency are also predisposed to having neutralizing IgG antibodies to factor IX that may be caused by shared factor IX gene defects, immune reaction genes, or both.¹⁶ These patients may also share a genetic predisposition for increased immunoglobulin isotype switching to IgE, which could be the explanation for the development of factor IX allergy in some patients with hemophilia B.

Desensitization to factor IX is crucial for the optimal management of patients with factor IX deficiency and inhibitors to factor IX who have allergic reactions to factor IX because it enables them to tolerate factor IX-containing activated PCCs. The activated PCCs are the only products currently licensed in the United States that are available for the treatment of acute bleeding episodes in factor IX-deficient patients with inhibitors. Because a more gradual desensitization protocol seems to be better tolerated, it would be best to consider desensitizing these patients electively and subsequently maintaining the desensitized state with daily or every other day infusions of factor IX. Although both of our patients received activated PCCs only after the full completion of the desensitization protocol, it is reasonable to postulate that the infusion of an activated PCC could potentially be tolerated uneventfully at the end of the first day of the desensitization protocol described in Table III after the cumulative dose of 100 U/kg of factor IX is reached. This could potentially be helpful in the treatment of acute bleeding episodes in patients allergic to factor IX who have not previously undergone desensitization to factor IX.

The presence of immediate-type allergic reactions to factor IX is a serious complication of hemophilia B therapy that severely limits the therapeutic options available in the management of hemophilic bleeding. Although older reports involved the use of impure mixtures of factor IX and other coagulation factors,^{1,2} more recent reports have

documented that allergic reactions occur even with the highly purified factor IX concentrates now available.^{3,4} Our experience with these two patients should be useful in the diagnosis and management of other patients with anaphylaxis or allergic reactions to factor IX.

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