

Case report

Anaphylaxis to subcutaneous neutral protamine Hagedorn insulin with simultaneous sensitization to protamine and insulin

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We report an insulin-treated diabetic patient who suffered, in a 2-month period, three severe anaphylactic reactions immediately after self-administered subcutaneous injections of neutral protamine Hagedorn (NPH) human recombinant-DNA insulin. These reactions consisted of local and systemic symptoms, including dyspnea and hypotension. A simultaneous sensitization to human insulin and to protamine was demonstrated, both by skin tests and by the determination of serum specific IgE. Suspecting protamine allergy, we performed a test dose to human lente insulin with perfect tolerance. After a 1-year follow-up with lente-insulin treatment, no reactions have occurred, despite treatment interruptions. Therefore, protamine IgE-mediated allergy probably caused our patient's reactions. In conclusion, protamine sensitization should be ruled out in any patient with a history of reactions to subcutaneous protamine-containing insulins, even if insulin sensitization is present.

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Insulin-requiring diabetic patients use either human recombinant DNA (rDNA) insulin or purified animal insulins. These animal insulins contain small amounts of noninsulin proteins that may be antigenic. Commercial insulins also contain additives such as zinc and protamine. Most patients regularly receiving exogenous insulins develop antibodies to these insulins, almost always directed to the insulin molecule, and not to the impurities or additives (1, 2). Clinical allergic reactions to insulin involve 5–10% of insulin-dependent diabetic patients (1). Most of these reactions are localized to the site of injection, and are mild and transient (1). Systemic anaphylactic reactions to insulin are uncommon, especially since new human recombinant DNA (rDNA) insulins are used, with a reported incidence of 0.1–2% (1). Reactions to human (rDNA) insulin are probably due to tertiary structure differences between endogenous insulin and synthetic insulin (3).

On the other hand, protamine sulfate, a polycationic protein derived from salmon milt, is a well-known cause of systemic reactions, during intravenous administration for reversal of heparin anticoagulation, in vascular surgery and cardiac catheterization (4, 5). Several mechanisms may be implicated, including IgE-mediated protamine allergy (4, 5). Protamine is also added to neutral protamine Hagedorn (NPH) insulin and protamine zinc insulin (PZI) to enhance stability. Diabetic patients receiving daily subcutaneous injections of insulin-containing protamine appear to have a 40- to 50-fold increased risk of life-threatening reactions when given protamine intravenously (4–6). However, to our knowledge, there have been only three reports of protamine as the cause of generalized allergic reactions to subcutaneous NPH insulin administration (2, 7, 8). We report the first case of anaphylaxis to subcutaneous human NPH insulin, in a patient with simultaneous sensitization to insulin and to protamine.

Case report

A 20-year-old insulin-dependent diabetic man had been treated with regular and NPH (rDNA) human insulins for 1 year without problems. He had a 10-year history of perennial rhinoconjunctivitis due to house-dust-mite allergy. In the 2-month period before admission, he had experienced three anaphylactic reactions 2–5 min after a self-administered subcutaneous injection of his morning dose of 16 U of NPH (rDNA) human insulin (Insulatard Novolet, Novo Nordisk SA, Denmark). A local reaction at the injection site with pruritus, wheal, and flare had been followed by generalized flushing, pruritus, dyspnea, and wheezing, requiring emergency room treatment. In the third episode, the reaction progressed to hypotension, and the patient was hospitalized for an evaluation of insulin allergy, which was performed 48 h after the reaction. Our patient had had some interruptions of insulin therapy, although no local reactions to insulins had occurred. There was no history of fish allergy or vascetomy.

Material and methods

Skin prick tests (SPT)

SPTs were carried out with regular, NPH, and lente human (rDNA) insulins (Novo Nordisk SA, Denmark) at a concentration of 40 U/ml, and with protamine sulfate at 10 mg/ml (Laboratorios Farmacéuticos Rovi SA, Madrid, Spain), by puncture with a Morrow-Brown needle (Bayer SA, Madrid, Spain) in the volar side of the forearms. Histamine phosphate (10 mg/ml) was used as positive control and physiologic saline as negative control. The results were expressed in the measurements (mm) of the larger wheal diameter 15 min after puncture. A wheal >3 mm in the presence of a negative response to the saline control was considered positive. Three atopic subjects and three NPH insulin-treated diabetic patients were used as a control group for *in vivo* tests. Written informed consent was obtained from all patients.

Intradermal skin tests (ID)

Intradermal testing was performed with serial 10-fold dilutions of insulins and protamine in sterile diluent, starting with a 1:100 000 dilution, reaching a maximum concentration of 0.4 U/ml for insulins and 0.1 mg/ml for protamine, and injecting 0.02 ml of the corresponding solution in the volar side of the forearms. Histamine phosphate (1 mg/ml) was used as positive control and physiologic saline as negative control. The results were seen in the measurements of the larger wheal diameter 20 min after

injection. A wheal over 5 mm larger than the saline control with erythema was considered a positive response. A positive ID test to one drug contraindicated the skin ID test with more concentrated solutions of this drug.

Total serum IgE

This was determined by the DELFIA method (Pharmacia Diagnostics, Sweden), following the manufacturer's instructions. Sera from two NPH insulin-dependent diabetic patients were used as controls for *in vitro* testing.

Specific serum IgE

Specific IgE to bovine, porcine, and human insulins, as well as to protamine, were assessed by the Pharmacia ImmunoCAP system, according to the manufacturer's instructions. Levels higher than 0.7 kU/l were considered positive. Due to technical limitations, specific IgE to protamine determination was performed 6 months after the initial study.

CAP inhibition

Aliquots (30 µl) of the patient serum were incubated for 1 h at room temperature, with 20 µl of 10-fold protamine or regular rDNA human insulin dilutions (100 µg/ml to 100 ng/ml) on phosphate buffer, pH 7.4, containing 0.03% (w/v) human albumin. ImmunoCAP to protamine (Pharmacia, Sweden) was then performed in duplicate, according to Pharmacia's directions for use. The degree of inhibition was measured in percentage, the zero level being defined as the uptake to the solid phase when allergen is replaced with phosphate buffer.

Results

Our patient's SPTs to regular, NPH, and lente human insulins were negative. SPT to protamine was positive, showing a 6-mm wheal with surrounding erythema. SPTs to protamine and insulins were negative in control subjects. ID to all insulins tested showed positive results in our patient (Table 1), being negative in controls. ID to protamine was positive in our patient at concentrations as low as 0.0001 mg/ml, being positive in two controls at 0.1 mg/ml. Skin tests were repeated in our patient 1 year after the initial study; ID tests were similar to the initial results, but SPT to protamine became negative. Our patient's total IgE was 70 kU/l (normal), and specific IgE results for insulin and protamine were positive (Table 2). During CAP to protamine inhibition, the preincubation of our patient's serum with dilutions of protamine as low

Table 1. Results of skin tests

Agent		Results (mm) wheal/flare (concentration)	
		Patient	Controls
Regular insulin	SPT	Neg	Neg
	ID	5/15 (0.004 U/ml)	Neg
NPH insulin	SPT	Neg	Neg
	ID	8/20 (0.004 U/ml)	Neg
Lente insulin	SPT	Neg	Neg
	ID	7/10 (0.004 U/ml)	Neg
Protamine	SPT	6/10 (10 mg/ml) [#]	Neg
	ID	9/20 (0.0001 mg/ml)	+/-*

[#] Repeated 1 year after initial study; yielded negative result.

* Two controls showed positive protamine ID tests at 0.1 mg/ml concentration.

Table 2. Results of specific IgE

	Patient	Controls
Porcine insulin	14.8 kU/l	Neg
Bovine insulin	3.6 kU/l	Neg
Human (rDNA) insulin	12.4 kU/l	Neg
Protamine	1.7 kU/l*	Neg

* Determined from serum obtained 6 months after initial study.

as 1:10000 resulted in 100% inhibition; however, the addition of regular insulin at concentrations as high as 40 UI/ml yielded no inhibition.

Because we considered insulin therapy clearly indicated in our patient, he was carefully test dosed to regular human insulin and to lente human insulin; he showed perfect tolerance, and he was discharged with one daily morning dose of lente human (rDNA) insulin. At 1-year follow-up, no new reactions had occurred, despite some proved treatment interruptions.

Discussion

Anaphylactic reactions to human (rDNA) insulin are rare. Most commonly, they occur after an interruption of insulin therapy, and after progressively larger local cutaneous reactions at the injection site (1). We report a patient with systemic anaphylactic reactions to NPH human (rDNA) insulin who presented several special features. First, we have demonstrated by skin tests and specific IgE the existence of simultaneous sensitization to both insulin and protamine. Second, there were several clinical characteristics not typical of insulin allergy: the severity of the reactions; the absence of previous local reactions, despite treatment interruptions; and the existence of refractory periods among the reactions, from 2 weeks to a few months in length, during which the patient tolerated subcutaneous NPH insulin injections without problems.

From these data, we suspected that insulin allergy

could be only an asymptomatic sensitization such as is frequently found in diabetic patients (1). In fact, most patients receiving insulin daily for a long time develop antibodies to it, and 40% of patients receiving insulin without clinical allergy develop positive immediate skin tests to the insulin being used for treatment (1, 2). Therefore, negative insulin cutaneous testing helps to exclude insulin allergy, but positive skin tests to insulin may have no diagnostic relevance (1). In our patient, the protamine sensitization could have caused the anaphylactic reactions. The perfect tolerance of regular and lente human insulins at the test dose performed, despite positive skin test and positive specific IgE to insulin, supports our hypothesis.

The fact that no reactions occurred in a 1-year follow-up (with daily lente human insulin, and with some treatment interruptions) also indicates protamine as the possible cause of the reactions. Moreover, two of the three patients previously described as allergic to subcutaneous protamine in insulin preparations share several clinical characteristics with our patient: the absence of previous local reactions, the severity of the reactions, and the refractory periods of some weeks or months between reactions (in which patients tolerate NPH insulin administration without problems, explained as temporary desensitization to protamine) (2, 7).

However, the value of skin tests with protamine is controversial (4, 5). Our patient showed a positive SPT to a 10-mg/ml protamine solution, as did one of the patients described by Dykewicz et al. (7), with negative results in controls. It is remarkable that in both cases the SPT was performed shortly after the reaction: this circumstance could have reduced skin reactivity due to the depletion of antiprotamine IgE. Intradermal skin tests with protamine have been reported to cause nonspecific positive responses at concentrations higher than 100 µg/ml (4, 5), as proved by our results in control patients. Our patient's positive response to intradermal protamine at 0.1 µg/ml strongly suggests IgE-mediated sensitivity. The presence of specific IgE to protamine was confirmed by CAP determination. The CAP inhibition procedure assessed the specificity of our patient's IgE antibodies against protamine determinants, although a dose-response curve could not be obtained due to his low level of specific IgE to protamine, a reduction which was probably related to IgE antibody declination over the time from the last exposure (5). Our data support the pathogenetic importance of IgE-mediated allergy in immediate-type reactions to protamine caused by subcutaneous administration of protamine insulin (7). In contrast, the different mechanisms implicated in reactions to intravenously administered protamine explain the

contradictory results found during protamine skin testing of patients with reactions to intravenous protamine (4, 5, 7).

Because skin tests to protamine are not routinely performed in determination of possible insulin allergy, and insulin skin tests frequently show false-positive results, we suggest that some patients with protamine allergy sensitized through subcutaneous NPH insulin administration could be misdiagnosed as allergic to insulin, and could continue to show clinical reactions if maintained with a protamine-containing insulin. Moreover, these patients would be at risk of severe anaphylactic reactions if they underwent vascular surgery or cardiac catheterization with protamine intravenous administration for reversal of heparin anticoagulation. We suggest that studies of any protamine-containing insulin reaction routinely include SPT with protamine at a concentration of 10 mg/ml, and ID tests with protamine at concentrations up to 0.01 mg/ml. In case of positive skin tests to protamine, patients should be test dosed to a regular and/or to a lente insulin, and informed of the high risk of future protamine intravenous or subcutaneous administration.

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References

1. Grammer L. Insulin allergy. *Clin Rev Allergy* 1986;4:189-200.
2. Kim R. Anaphylaxis to protamine masquerading as an insulin allergy. *Del Med J* 1993;65:17-23.
3. Grammer L, Metzger BE, Patterson R. Cutaneous allergy to human (rDNA) insulin. *JAMA* 1984;251:1459-60.
4. Weiler JM, Gellhaus MA, Carter JG, et al. A prospective study of the risk of an immediate adverse reaction to protamine sulfate during cardiopulmonary bypass surgery. *J Allergy Clin Immunol* 1990;85:713-19.
5. Weiss ME, Adkinson NF Jr. Allergy to protamine. *Clin Rev Allergy* 1991;9:339-55.
6. Stewart WS, McSweeney SM, Kellet MA, Faxon DP, Ryan TJ. Increased risk of severe protamine reactions in NPH insulin-dependent diabetics undergoing cardiac catheterization. *Circulation* 1984;70:788-92.
7. Dykewicz MS, Kim HW, Orfan N, Yoo TJ, Lieberman P. Immunologic analysis of anaphylaxis to protamine component in neutral protamine Hagedorn human insulin. *J Allergy Clin Immunol* 1994;93:117-25.
8. Sanchez MB, Paolillo M, Chacon RS, Camejo M. Protamine as a cause of generalized allergic reactions to NPH insulin [Letter]. *Lancet* 1982;1:1243.

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