

Immunologic analysis of anaphylaxis to protamine component in neutral protamine Hagedorn human insulin

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Abstract

We report the clinical and immunologic analysis of two patients with diabetes who had anaphylaxis to neutral protamine Hagedorn (NPH) human insulin in the absence of allergy to regular insulin. A 36-year-old woman without a recent history of local insulin reactions or interruption of insulin therapy experienced anaphylaxis within 15 minutes of her usual morning dose of subcutaneously administered NPH human insulin. A 62-year-old man with a history of generalized reactions to NPH human insulin and of anaphylaxis to intravenously administered protamine had generalized urticaria after injection of NPH human insulin. Both patients subsequently tolerated Lente human insulin. Skin test results in both patients were negative to regular and Lente insulin preparations but positive to NPH insulin and to protamine at concentrations tested. In vitro assays demonstrated that both patients had markedly elevated serum levels of IgE and IgG to protamine, but not to regular human insulin, and that their IgE antibodies to protamine recognized protamine antigenic determinants in NPH human insulin. We conclude that the anaphylactic reactions to NPH insulin in our patients were mediated by IgE to protamine, which should be a pathogenetic consideration in the evaluation of immediate-type reactions to protamine-containing insulins. (J ALLERGY CLIN IMMUNOL 1994;93:117-25.)

NPH: Neutral protamine Hagedorn(insulin)

Key words: [Insulin](#), [diabetes](#), [protamine](#), [immediate-type reactions](#), [anaphylaxis](#)

It is well recognized that immediate-type, systemic reactions to bovine, porcine, and human insulin preparations may occur through type I IgE-mediated mechanisms. ¹⁻¹¹ Insulin preparations used for management of diabetes mellitus may contain not only insulins (i.e., regular insulins) but also other components such as zinc (in most available insulin preparations) or protamine (in neutral protamine Hagedorn [NPH] insulins and PZI), which are added to enhance stability or alter duration of pharmacologic effect. However, IgE antibody to insulin antigenic determinants or, before the availability of highly purified insulins, to animal protein impurities from animal insulin sources has been demonstrated to be responsible for most immediate-type reactions to insulin preparations. ^{1,2}

Protamines, polycationic proteins (4500 d) purified commercially from salmon milt, are used not only in insulin preparations, but are also administered intravenously to reverse heparin anticoagulation. Intravenous administration of protamine can cause acute reactions that may include rash, urticaria, transient elevations in pulmonary artery pressure, bronchospasm, hypotension, cardiovascular collapse, and death. ¹²⁻¹⁹ These adverse reactions are thought to be caused by multiple putative mechanisms including IgE-mediated reactions ^{12-14, 20}; activation of complement (either by protamine-heparin complexes or through interaction with protamine-anti-protamine IgG antibody complexes) leading to generation of anaphylatoxins C3a, C4a, and C5a with subsequent mast cell and basophil activation ²¹⁻³⁰; direct, nonimmunologic histamine release from mast cells ³¹; inhibition of serum carboxypeptidase ³²; potentiation of IgE-mediated histamine release ³³; and elevation in thromboxane B₂ and 6-keto-prostaglandin F₁ α levels causing pulmonary artery pressure elevation. ³⁴⁻³⁸ Although the reported incidence of intravenous protamine reactions varies, it is clear that patients with diabetes who have a history of use of protamine-containing insulins are at significantly increased risk for reactions to in-travenously administered protamine; such reactions occur in 2.9% to 26.6% of these patients. ^{12, 18, 19, 39, 40} In contrast, only two patients have been reported to have had immediate-type reactions to subcutaneous NPH insulin administration caused by apparent sensitivity to protamine or to either protamine or zinc. ^{41, 42} However, serologic studies were not performed in these patients, and concentrations of agents used for skin testing may have caused nonspecific, irritant responses. There has been a report of delayed local reactions to NPH insulin, which were thought to be caused by protamine sensitivity. ⁴³

We report the clinical and immunologic analysis of two patients who had immediate-type, systemic reactions to NPH human insulins in the absence of allergy to regular human insulin.

Case Histories

Patient 1

A 36-year-old woman with diabetes mellitus had been treated with insulin preparations for more than 10 years. Although she had a history of local, immediate-type reactions to bovine and porcine insulin preparations, she had been treated with regular and NPH human insulin for approximately 3 years without experiencing any local or generalized immediate-type reactions. There was no history of fish allergy. Approximately 5 minutes after a self-administered, subcutaneous injection of her normal morning dose of 22 U of NPH human insulin (Eli Lilly, Indianapolis, Ind.), a local wheal and flare reaction developed at the injection site on the abdomen, which was followed by the development of generalized urticaria, shortness of breath, and light-headedness. The patient was then hospitalized for evaluation of insulin allergy and for insulin therapy.

That afternoon, results of immediate-type skin tests to regular human, porcine, and bovine insulin were negative (■Table I). Skin test results were positive to NPH human, porcine, and bovine-porcine insulins but were negative to Lente human insulin at concentrations tested. Skin prick test results to protamine were negative at 1 mg/ml but positive at 10 mg/ml.

TABLE I. Immediate-type skin testing

Concentrations tested and results (mm wheal/erythema)		
Agent	Patient 1	Patient 2
Regular insulin	(Tested human, porcine, & bovine) – scratch up to 100 U/ml – ID up to 1 U/ml	(Tested bovine-porcine) – Scratch up to 10 U/ml – ID up to 10 U/ml
NPH insulin		
Human	+ ID (5/19 mm) at 0.01 U/ml	
Porcine	+ ID (7/16 mm) at 0.01 U/ml	
Bovine-porcine	+ ID (7/13 mm) at 0.01 U/ml	– Scratch up to 10 U/ml + ID (10/20 mm) at 0.1 U/ml
Protamine sulfate	– Scratch at 1 mg/ml + Scratch (4/18 mm) at 10 mg/ml	– Scratch up to 1 mg/ml + ID (15/30 mm) at 1 µg/ml
Lente insulin	(Tested human) – Scratch up to 100 U/ml – ID up to 1 U/ml	(Tested bovine-porcine) – Scratch up to 10 U/ml – ID up to 10 U/ml

Both patients were tested with pure insulin preparations from Eli Lilly (Indianapolis, Ind.). Protamine sulfate used for patient 1 was from Lilly and for patient 2 from Lyphomed, Inc. (Rosemont, Ill.).
ID, Intradermal.

The patient was subsequently test dosed to Lente human insulin, which was tolerated without adverse effect and was used successfully with regular human insulin for control of diabetes.

Patient 2

A 62-year-old man with diabetes mellitus had been treated with Lente human insulin (Lilly) for several years without difficulty. During a viral infection, his diabetes became poorly controlled and his insulin preparation was changed to Novolin 70/30 (70% NPH human insulin, 30% regular human insulin [Novo Nordisk, Princeton, N.J.]) given in divided doses. Subsequently, local pruritus began to develop at injection sites, and generalized flushing occurred within 5 minutes of each insulin injection. Within several weeks, reactions became more severe and included wheezing, shortness of breath, chest pain, and finally a syncopal episode. At the time of the syncopal episode, the patient's blood glucose level was 98 mg/dl, and results of subsequent diagnostic studies including cardiology workup were unremarkable. Because it was hypothesized that the syncopal episode was a hypoglycemic reaction, his Novolin 70/30 dose was reduced but he continued to have mild itching and flushing after each injection. Several weeks later after another syncopal episode occurred, Novolin 70/30 insulin was discontinued and was replaced with NPH insulin (Lilly), which was tolerated by the patient for several weeks without significant reaction. He was then hospitalized for elective bifemoral bypass surgery. During surgery, he received protamine intravenously to reverse heparin anticoagulation. Within a minute of protamine administration, anaphylactic shock including hypotension and respiratory distress developed, which required aggressive resuscitative measures including intravenous administration of epinephrine and fluids and intubation with mechanical ventilation for 3 days. While in the hospital, his diabetes was managed with regular human insulin. After discharge, NPH human insulin was resumed and was well tolerated for approximately 3 weeks until local pruritus and urticaria developed, which was followed by generalized urticaria within several minutes of a subcutaneous abdominal injection of his usual morning insulin dose.

The patient was then referred for allergy evaluation. Skin test results were negative to regular bovine-porcine insulin, positive to NPH bovine-porcine insulin, positive to protamine, and negative to Lente bovine-porcine insulin at the concentrations indicated in ■Table I. The patient was then test dosed and treated successfully with Lente human insulin. There was no history of vasectomy, infertility, or fish allergy.

Methods

Subjects and serum samples

Serum samples were obtained from patient 1 1 day after she experienced anaphylaxis and from patient 2 on the day of his generalized reaction when he was referred for allergy evaluation. Control sera for ELISA assays for IgE to human regular insulin were obtained from a patient with a known history of a generalized systemic reaction to human insulin ¹¹ and a pool of sera from eight normal control subjects without a history of exogenous insulin use. Written informed consent was obtained from control subjects under a protocol approved by the Institutional Review Board of Saint Louis University.

RASTs for serum IgE and IgG to protamine

Serum levels of protamine-specific IgE and IgG antibodies were determined by RAST performed by the Johns Hopkins Medical Institutions DACI Reference Laboratory, Baltimore, Md. Soluble protamine inhibition was performed by the laboratory to assess the specificity of the antibody detected.

Inhibition studies of ELISA for IgE to protamine

To establish whether serum IgE antibodies to protamine recognized protamine antigenic determinants in NPH insulin, inhibition studies of an ELISA for IgE to protamine were performed according to ELISA methods previously described ⁴⁴ with the addition of a serum preincubation step before serum addition to microtiter plates. Briefly, the following reagents were added in sequence to Immulon II microELISA plates (Greiner & Sons, Nürtingen, Germany) with appropriate washes and incubation periods: protamine sulfate (Quad Pharmaceuticals, Inc., Indianapolis, Ind.) in carbonate coating buffer; dilutions of serum samples preincubated at 37° C for 30 minutes with dilutions of either (1) protamine sulfate, (2) NPH human insulin, (3) regular human insulin, or (4) saline solution; rabbit anti-human IgE (Accurate Chemical & Scientific Corporation, Westbury, N.Y.), alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, Mo.), and p-nitrophenyl phosphate (Sigma). Optical density of wells was then read at 405 nm on an automated ELISA reader.

In vitro assays for IgE to regular insulin

Serum levels of human IgE to regular human insulin for both patients were determined by ELISA according to methods previously described. ¹¹ Briefly, the following reagents were added in sequence to Immulon II microELISA plates with appropriate washes and incubation periods: regular human insulin, in carbonate coating buffer, rabbit anti-human IgE, alkaline phosphatase-conjugated goat anti-rabbit IgG, and p-nitrophenyl phosphate. Optical density of wells was then read at 405 nm.

Skin testing of normal control subjects with NPH human insulin

To determine whether concentrations of NPH insulin used for skin testing caused nonspecific reactions, 0.02 ml of dilutions of NPH insulin were injected intradermally in seven normal subjects without history of exposure to protamine or protamine-containing insulins. Subjects were also simultaneously scratch tested with histamine phosphate 5 mg/ml (Allermed Laboratories, Inc., San Diego, Calif.) as a positive control. Wheal and flare responses were assessed at 20 minutes after injection.

Results

RASTs for serum IgE and IgG to protamine

As indicated in ■Table II, serum levels of antibodies to protamine were significantly elevated for both patient 1 (IgE, 9 ng/ml with 95% inhibition with protamine; IgG, 978 ng/ml with 85% inhibition) and patient 2 (IgE, 24 ng/ml with 98% inhibition; IgG, 1163 ng/ml with 21% inhibition). In these assays normal subjects have levels of anti-protamine IgE less than 1 ng/ml (Robert G. Hamilton, PhD, Johns Hopkins Medical Institutions DACI Reference Laboratory; oral communication, September 1992) and of anti-protamine IgG less than 500 ng/ml.²⁰ For comparison, ■Table II also summarizes serum antibody results for patients who did and did not have reactions to intravenous protamine administration with the same RAST assays for IgE and IgG to protamine and an ELISA for serum IgG to protamine as reported by Weiss et al.^{20, 45} The serum level of anti-protamine IgE of patient 1 is similar to and that of patient 2 is higher than that of a patient reported to have had anaphylaxis to intravenously administered protamine.⁴⁵ The levels of anti-protamine IgG for patients 1 and 2 were in the range reported for some patients who had reactions to intravenously administered protamine, although patients with diabetes and a history of NPH insulin use who had reactions to intravenously administered protamine had much higher IgG levels (median, 36,400 ng/ml).²⁰

TABLE II. Serum levels of IgE and IgG to protamine in patients with reactions to protamine component in NPH insulin compared with patients with and without reactions to intravenous protamine administration

	IgE ng/ml (RAST)	IgG ng/ml (RAST, ELISA)
Reactors to NPH Insulin (this report)		
Patient 1	9	978‡
Patient 2	24	1163‡
Reactors to protamine administration (Weiss et al.) ^{20, 45}		
Anaphylaxis: acute/1 month after reaction ⁴⁵ (diabetic with history of NPH insulin use)	8.5/16	1300/90500‡
Diabetics with history NPH insulin use (n= 13) ²⁰	n = 9 ≥1 ng/ml* n = 4 < 1 ng/ml*	36400§ median, ≥500 ng/ml† in n = 11
Patients without history of NPH insulin use (n= 14) ²⁰ (13 normal, 1 diabetic)	All < 1 ng/ml*	800 [§] median ≥500 ng/ml† in n = 5
Nonreactors to protamine administration (Weiss et al.) ²⁰		
Diabetics with history NPH insulin use (n= 22) ²⁰	All < 1 ng/ml*	100 [§] median, ≥500 ng/ml† in n = 2
Diabetics, no history NPH insulin use (n= 21) ²⁰	All < 1 ng/ml*	All < 500 ng/ml†§
Normal subjects, no prior protamine ²⁰ (n = 13 for IgE, n = 10 for IgG)	All < 1 ng/ml*	All < 500 ng/ml†§

*Positive level of IgE to protamine defined in original report as counts per minute 2.5 times or greater than that of negative serum samples and inhibited 50% or more by soluble protamine. This level has now been determined by Johns Hopkins DACI Laboratory as approximately ≥1 ng/ml (personal communication, Robert G. Hamilton, PhD). In original publication, data were expressed in terms of RAST binding ratios.

†Positive level of IgG to protamine by ELISA defined in original report as ≥500 ng/ml that was inhibited ≥ 50% by protamine.²⁰

‡Results determined by RAST.

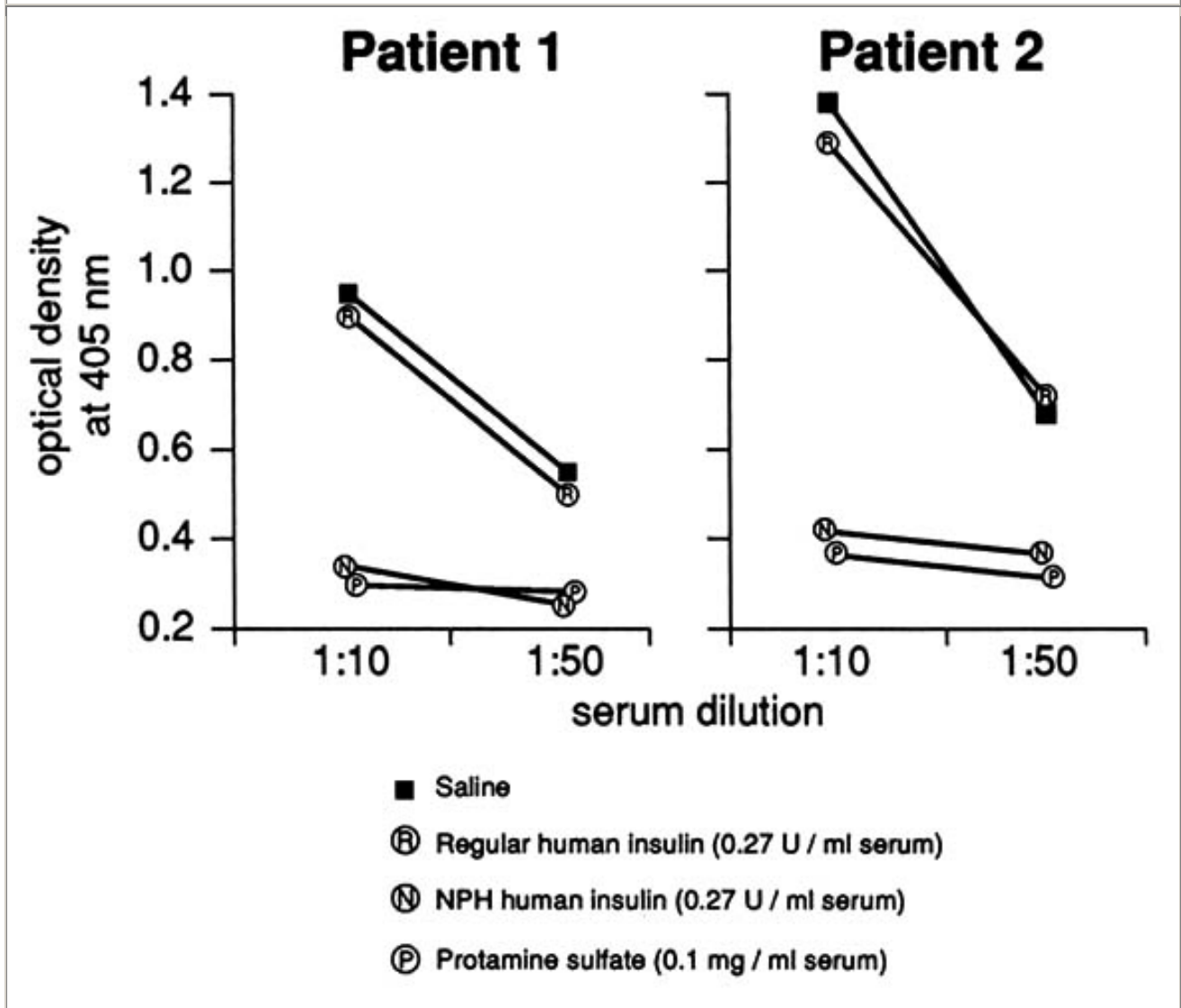
§Results determined by ELISA. (Changes were made in this electronic version for 800[§] and 100[§] median, per "Correction" printed in J ALLERGY CLIN IMMUNOL 1994;93:810.)

Inhibition studies of ELISA for IgE to protamine

Results of ELISA inhibition studies are shown in ■Fig. 1. The ELISA for IgE to protamine was significantly inhibited by preincubation of sera from both patients with protamine and NPH human insulin, but not with

regular human insulin, demonstrating that protamine determinants in NPH insulin were recognized by the serum anti-protamine IgE antibody detected.

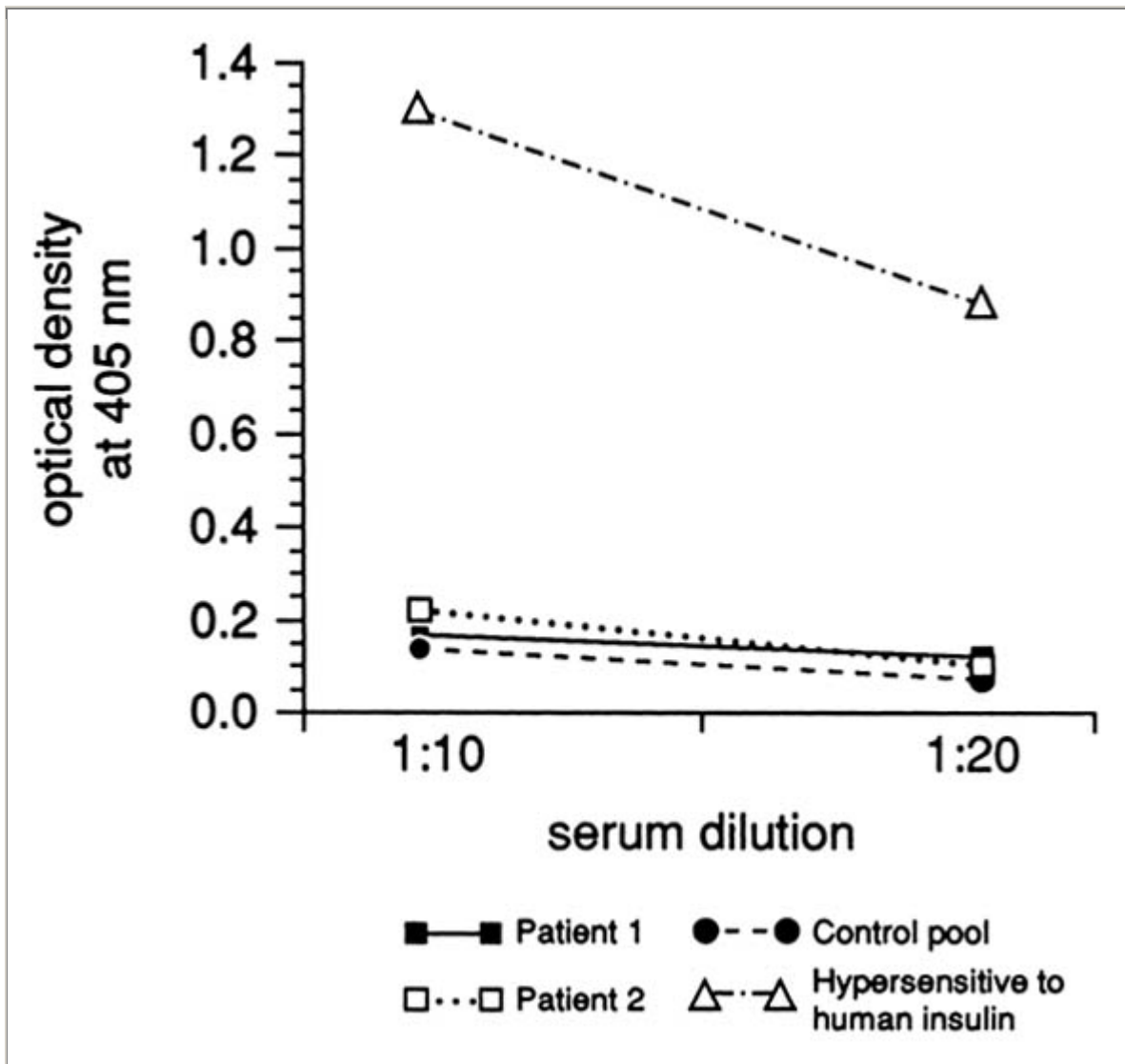
FIG. 1. Inhibition studies of ELISA for IgE to protamine in serum of the patients in this report. Results shown are for serum coincubations with agents indicated.



In vitro assays for IgE to regular insulin

In comparison to serum from a patient with known allergy to human insulin and pooled sera from normal control subjects (negative control sera), neither patient 1 nor patient 2 had elevated serum levels of IgE to regular human insulin as determined by ELISA (■Fig. 2).

FIG. 2. Serum levels of IgE to regular human insulin as determined by ELISA.



In addition, serum from patient 2 was also reported to be negative for IgE to regular bovine, porcine, and human insulin by RAST performed by the Johns Hopkins Medical Institutions DACI Reference Laboratory.

Skin testing of normal control subjects with NPH human insulin

No normal subjects had wheal and flare responses to NPH human insulin skin tests at concentrations of 1 U/ml with simultaneous positive histamine control skin test results (wheal > 5 mm with flare).

Discussion

The in vitro and clinical data presented indicate that IgE-mediated reactions to the protamine component in NPH insulin were responsible for the generalized, immediate-type reactions that occurred after NPH insulin administration in both patients in this report. In vitro evidence supporting this conclusion are (1) the presence of significantly elevated serum levels of IgE to protamine, (2) demonstration by ELISA inhibition studies that serum IgE antibodies to protamine in our patients recognized protamine antigenic determinants in NPH insulin, and (3) the absence of elevated IgE levels against regular human insulin.

Skin test results of both patients are consistent with in vitro antibody studies. The absence of skin test reactivity to regular insulin preparations in our patients confirms the negative results of the in vitro studies for IgE antibody to regular insulin and effectively eliminates the possibility that our patients had IgE-mediated reactions against insulin antigenic determinants, particularly because skin test reactivity is generally more

sensitive in detecting IgE antibody than are in vitro tests.⁴⁶ Because intradermal testing with protamine sulfate causes nonspecific reactions at higher concentrations,¹² it was important to determine whether the concentrations of NPH insulin that caused wheal and flare skin responses in our patients did so by nonspecific, irritant effects to protamine. Because we established that an intradermal skin test of 1 U/ml NPH human insulin did not cause wheal and flare responses in normal control subjects, we believe that the positive skin test results to NPH insulins in patient 1 at a concentration of 0.01 U/ml and to NPH bovine-porcine insulin in patient 2 at a concentration of 0.1 U/ml do not represent nonspecific responses. Moreover, on the basis of an average content of 0.3 to 0.5 mg protamine per 100 U of NPH insulin, the NPH insulin 1 U/ml solution that we tested intradermally in normal subjects contains only 3 to 5 µg/ml protamine, and the NPH insulin concentrations that caused wheal and flare reactions in our patients contain only 0.03 to 0.05 µg/ml (patient 1) and 0.3 to 0.5 µg/ml (patient 2) protamine, well below 100 µg/ml protamine, which is the threshold concentration reported to cause nonspecific intradermal responses.^{12, 45} Because skin test results to the corresponding regular insulins were negative in our patients and because protamine is the only component present in NPH insulins that is absent in corresponding regular insulins (both preparations contain zinc), insulin skin test results in our patients indicate IgE-mediated immunoreactivity against protamine capable of inducing cutaneous mast cell mediator release. Although it is possible that the positive response in patient 1 to the 10 mg/ml protamine scratch test was a nonspecific, irritant response, the positive response in patient 2 to the 1 µg/ml protamine intradermal test was below the established intradermal irritant threshold and is further evidence of an IgE-mediated response to protamine. These positive skin test results are particularly impressive inasmuch as they were performed within hours of generalized reactions, which could have reduced skin reactivity because of the depletion of anti-protamine IgE or preformed mast cell mediators.

Weiss et al.⁴⁵ have reported that patients may have reactions to intravenously administered protamine with detectable levels of serum IgE antibody to protamine, yet have negative skin test results to protamine and equivocal in vitro basophil histamine release to protamine. They suggest that one explanation for this discrepancy could be that protamine may be an incomplete or univalent antigen and must first interact with a tissue macromolecule to become a complete or multivalent antigen capable of eliciting IgE-dependent mediator release.⁴⁵ In the case of skin tests or clinical reactions to protamine insulins, a tissue macromolecule may be unnecessary because protamine could interact with insulin to become a complete or multivalent antigen.

Although our data clearly demonstrate the presence of IgE antibody to protamine in our patients, studies of reactions to intravenously administered protamine have suggested that in some circumstances, serum IgE to protamine may be present only as an epiphenomenon and may not be causally related to development of reactions.⁴⁵ There is good evidence for a role of IgE to protamine in the pathogenesis of some reactions to intravenously administered protamine, however. Although Weiss et al.²⁰ reported that patients in their series who had a history of protamine-containing insulin use were at increased risk for reactions to intravenously administered protamine even if they have no detectable levels of antibodies to protamine, patients who also had IgE to protamine were at highest risk for reactions (relative risk = 95) and all of their reactions were moderate to severe. The presence of IgG antibody to protamine was a significant but less important risk factor (relative risk = 38). Patients who did not have IgE antibody to protamine either did not have reactions or tended to have less severe reactions, although such reactions could be severe. In our patients who had systemic reactions to subcutaneous administration of protamine-containing insulin, the pathogenetic importance of IgE to protamine detected by in vitro tests and skin tests is supported by the clinical observation that our patients had local reactions at the injection site of NPH insulin when their generalized reactions developed. We do not believe it likely that IgG antibody to protamine, which could cause complement activation, or other non-IgE-mediated mechanisms, which are suspected of causing reactions to protamine administered intravascularly could elicit the immediate local reactions at NPH insulin injection sites that were observed in our patients. Because of differences in dose and route of administration, subcutaneous administration of protamine insulin (which contains only one hundredth to one thousandth of the usual protamine dose used intravascularly for heparin neutralization) may be incapable of eliciting putative non-IgE-mediated mechanisms responsible for reactions to intravenously administered protamine. In the series of Weiss et al.²⁰ none of the patients with diabetes who had reactions to intravenously administered protamine reported reactions to their daily subcutaneous protamine insulin injections. They noted that this supports the concept, as described in patients who are allergic to penicillin, that the development of adverse drug reactions depends in part on the dose and the route of administration. This may explain why immediate-type reactions to protamine caused by subcutaneous administration of protamine insulins are apparently unusual.

Clinically, the histories of our patients are atypical of patients who have immediate-type reactions to insulin. Usually, patients who have generalized reactions to insulin have a history of increasingly large local reactions to insulin injections before the development of generalized reactions and a history of interruption of insulin therapy. ^{1,2} In contrast, patient 1 had no interruption of protamine insulin therapy and no recent history of local reactions to NPH insulin injections before the development of anaphylaxis, although she did have a distant history of local reactions to animal insulin preparations. Although patient 2 did have a history of local reactions to NPH insulins before the development of generalized reactions, the generalized urticarial reaction that prompted his referral to an allergist occurred after several weeks of tolerating NPH human insulin without apparent local reactions. However, the patient may have been temporarily "desensitized" to the protamine in NPH insulin because of his anaphylaxis to intravenously administered protamine. His last generalized reaction did follow a perioperative interruption of protamine insulin therapy when he was treated with regular insulin, but his first systemic reactions to NPH insulins (which in retrospect were likely reactions to the protamine in the insulin preparations) were not preceded by an interruption of protamine insulin treatment.

Our patients also appear to be unusual in that they had putative IgE-mediated anaphylaxis to a drug during a continuous course of therapy with that drug. We speculate that our patients may have been producing increasing amounts of IgE to protamine in response to subcutaneous NPH insulin injections, but anaphylaxis did not occur because protamine sequestered in NPH insulin suspensions at local injection sites was released into the general circulation in a slow and gradual manner, essentially analogous to desensitization. However, on the days that anaphylaxis occurred, a larger than usual amount of protamine may have been rapidly released or introduced intravascularly, resulting in rapid, generalized degranulation of mast cells or basophils that caused anaphylaxis. This may also be an additional explanation why some patients with diabetes can receive subcutaneous NPH insulin without apparent reaction after development of IgE to protamine, yet have putative IgE-mediated anaphylaxis only after intravenous administration of protamine.

Although it has been suggested that fish allergy, and in men, vasectomy and infertility are possible risk factors for protamine sensitivity, ^{47,48} our patients had no history of these. Moreover, it has never been convincingly demonstrated that there is an association between fish allergy and protamine sensitivity, and there is in vitro evidence for a lack of immunologic cross-reactivity between salmon and protamine. ⁴⁴

These case reports demonstrate the importance of considering protamine sensitivity when patients have local or systemic reactions to protamine insulins such as NPH insulin or PZI. In the vast majority of cases, experience indicates that reactions to protamine insulins are mediated by sensitivity to insulin determinants. In clinical evaluation of immediate-type reactions to protamine insulins, negative skin test results to regular insulins (with a positive control skin test result) would make it likely that sensitivity to protamine is present and therefore should prompt skin testing to NPH insulin, which we have demonstrated may be performed intradermally at concentrations of 1 U/ml or less without nonspecific reaction. This recommendation for skin test concentrations may not apply for PZI, which contains more protamine (1 to 1.7 mg protamine per 100 U insulin) than does NPH insulin (0.3 to 0.5 mg protamine per 100 U insulin), ¹² and therefore may be more prone to cause nonspecific positive skin test results at high concentrations. If skin test results to NPH insulin are positive in the absence of regular insulin skin test reactivity, consideration should be given to use of Lente series insulins (Semilente, Lente, Ultralente), which do not contain protamine but provide prolonged pharmacologic effect. Positive skin test results to regular insulin may not preclude the presence of concomitant protamine sensitivity of clinical importance, particularly because 40% to 50% of patients with diabetes may have positive skin test results to insulin, most in the absence of clinical allergy to insulin. ^{1,49} In such circumstances, comparison of the wheal and flare skin responses to regular and NPH insulins might reveal greater reactivity to NPH insulin, which would suggest protamine sensitivity. Consideration may also be given to intradermal testing with protamine sulfate, not to exceed 10 µg/ml (a nonirritant concentration established by Weiss et al.). ⁴⁵ Patients with diabetes identified as having clinical sensitivity to protamine insulin would appear to be at high risk for life-threatening reactions from intravenous protamine administration, and alternative approaches such as autoreversal of heparinization or use of hexadimethrine ⁵⁰ should be considered when heparinization reversal is required for such patients.

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