

INSTRUCTIVE CASE REPORT

Serial immunological investigations in a patient who had a life-threatening reaction to intravenous protamine

M. E. WEISS, F. CHATHAM*, ANNE KAGEY-SOBOTKA
and N. F. ADKINSON JR

*Johns Hopkins University School of Medicine, Division of Clinical Immunology at the Johns Hopkins Asthma and Allergy Center and *Franklin Square Hospital, Department of Internal Medicine, Baltimore, U.S.A.*

Summary

Reactions to intravenous protamine include rash, urticaria, bronchospasm, hypotension, and/or pulmonary artery pressure elevation. We have previously shown that in diabetic patients receiving daily protamine–insulin injections, the presence of anti-protamine IgE or IgG antibodies are significant risk factors for acute, life-threatening reactions when protamine is given intravenously. To study protamine reactions further, we measured serum anti-protamine IgE and IgG antibody levels, *in-vitro* basophil histamine release and intracutaneous skin testing to protamine serially in an NPH-insulin dependent diabetic who had a severe, protracted anaphylactic reaction to protamine. At the time of his protamine reaction, his serum contained 8.5 ng/ml of anti-protamine IgE and 1.3 µg/ml of anti-protamine IgG antibody. One month following the reaction both anti-protamine IgE and IgG increased to 16 ng/ml (twofold rise) and 90.5 µg/ml (70-fold rise), respectively. With time, both anti-protamine IgE and IgG antibody declined. Serial intradermal skin tests using protamine sulphate did not discriminate between the protamine reactor and nine normal control subjects who had no prior exposure nor any demonstrable serum IgE antibody to protamine. *In-vitro* basophil histamine release to protamine sulphate was inconclusive in discriminating between the protamine reactor and normal control subjects. We postulate that protamine may be an incomplete or univalent antigen that must first combine with a tissue macromolecule or possibly heparin to become a complete multivalent antigen capable of eliciting IgE antibody-dependent mediator release.

Clinical and Experimental Allergy, Vol 20, pp. 713–720. Submitted 5 January 1990; revised 26 March 1990; accepted 5 April 1990.

Introduction

The use of intravenous protamine to reverse heparin anti-coagulation has increased in the last decade with the advent of cardiopulmonary bypass technology, cardiac catheterization, haemodialysis, and phoresis. During this period, reports of adverse reactions associated with intravenous protamine administration (rash, urticaria, bronchospasm, hypotension, and/or elevated pulmonary artery pressure) have also increased [1–11].

The exact mechanism(s) by which protamine pro-

duces its adverse reactions is incompletely understood at present. It has been suggested that protamine-induced reactions are caused by direct, non-immunological histamine release from mast cells [12], complement activation via protamine–heparin complexes [13–16] or via protamine–anti-protamine IgG antibody interaction [8–10], inhibition of serum carboxypeptidase [17], thromboxane production [5,18–21], and anti-protamine IgE antibody via type I immediate hypersensitivity reactions [1,2]. It has also been suggested that protamine can potentiate IgE-dependent basophil histamine release through a polycationic recognition site on the cell membrane [22]. There is reasonable evidence that reactions involving an elevation of pulmonary artery pressure may have a different mechanism than those associated with

Correspondence: Dr N. Franklin Adkinson Jr, Johns Hopkins University School of Medicine, The Johns Hopkins Asthma and Allergy Center, 301 Bayview Boulevard, Baltimore, MD 21224, U.S.A.

bronchospasm and/or hypotension without pulmonary vasoconstriction [11,18].

Diabetic patients receiving daily subcutaneous injections of protamine-insulin preparations have a 40- to 50-fold increased risk of reactions when given protamine intravenously [6,7]. We have shown that in protamine-insulin-dependent diabetics, the presence of anti-protamine IgE or IgG antibodies is a significant risk factor for acute protamine reactions [11]. To define the immunological mechanism underlying protamine reactions better, we measured anti-protamine IgE and IgG serum levels, *in-vitro* basophil histamine release, and intradermal skin tests to protamine serially in an NPH insulin-dependent diabetic who had a severe, protracted anaphylactic reaction to intravenous protamine.

Case report

A 69-year-old white male with a 10-year history of diabetes mellitus and neutral protamine Hagedorn (NPH) insulin use (27 units s.c. q AM) underwent cardiac catheterization to evaluate sub-sternal chest pains. Heparin (8700 units) was given after cannulation of the right femoral artery. Selective injections of Renografin-76 were made without difficulty. After completion of catheterization, slow infusion of protamine sulphate (76 mg) diluted in D5W (50 ml) was begun. After 9 ml (14 mg of protamine sulphate) the patient developed progressive, severe hypotension (systolic blood pressure 150–60 mmHg), associated with tachycardia (heart rate = 110), dyspnoea (respiratory rate = 22), erythematous rash, and pruritus. The patient was immediately treated with i.v. diphenhydramine (total of 100 mg) and i.v. epinephrine (1 mg $\times$ 7 doses). Bolus i.v. epinephrine only temporarily corrected the hypotension and the patient became somnolent and was incubated. He was placed on epinephrine by a continuous infusion (2–3 mg/hr) to maintain a systolic blood pressure greater than 90 mmHg. The patient was treated with intravenous fluids, steroids (hydrocortisone 200 mg i.v. initially, then methylprednisolone 40 mg i.v. 6 hr), diphenhydramine (50 mg i.v. every 6 hr) and ranitidine (50 mg i.v. every 6 hr). Over the next 40 hr, repeated attempts to wean the patient from the epinephrine infusion were unsuccessful because of recurrent hypotension. Approximately 48 hr after the reaction, the patient was successfully weaned from the epinephrine infusion. Cardiac enzymes were compatible with a small sub-endocardial infarction (CPK-211 U/l with 5.2% MB bands; normals being 0–200 and 0–5% respectively). The patient had a full recovery and was switched from NPH to lente insulin. The patient denied a previous history of atopy and had a total IgE level of 135 ng/ml.

Materials and methods

Solid-phase radioimmunoassays for anti-protamine IgE and IgG

Protamine sulphate (Eli Lilly & Co., Indianapolis, U.S.A.) was insolubilized to cyanogen bromide-activated Sepharose CL-4B beads (Pharmacia, Piscataway, U.S.A.) as previously described [23] and IgE antibody to protamine was measured using a standard agarose-based radio-allergosorbent test (RAST) [23].

Anti-protamine IgG antibody was determined by means of a modification of RAST employing [^{125} I] *Staph* protein A [24–26]. Following a pre-absorption step to bind all naturally occurring anti-agarose antibodies [27], the assay was performed in a manner identical to the RAST, except that [^{125} I] *Staph* protein A was substituted for [^{125} I] anti-human IgE. Inhibition of anti-protamine IgE and IgG antibody binding to the protamine-agarose complex was evaluated by pre-incubating sera with increasing concentrations of soluble protamine sulphate for 1 hr before addition to the protamine solid phase.

Standardization of the anti-protamine IgE and IgG antibody in the reference serum

Absolute measurement of anti-protamine IgE antibody in the serum of the protamine reactor (described above) was performed using the antibody depletion (immunoabsorption) method described by Gleich *et al.* [28,29]. Briefly, 300 μ l of 20% (v/v) protamine-agarose complex or agarose beads were added to 300 μ l serum. The samples were rotated orbitally overnight at 25°C followed by a brief centrifugation (900 $\times$ g) to sediment the sorbent. An aliquot of each supernatant was then evaluated for total IgE content using a direct (non-competitive) RAST described previously [23]. The assay is sensitive to ≤ 1 ng/ml and has a mid-range coefficient of variation of 5%. An aliquot of each supernatant was also evaluated in the protamine RAST (described above) using unabsorbed serum from the protamine reactor to generate a standard curve. This was carried out to determine the fraction of protamine IgE antibody removed during the prior protamine-agarose or agarose (sham) incubation. Anti-protamine IgE antibody in the protamine reactor's serum is equal to the total IgE antibody in the supernatant after absorption with agarose minus the total IgE in the supernatant after absorption with protamine-agarose, after correcting for the fraction of protamine-specific IgE antibody removed during the protamine-agarose absorption.

The concentration of anti-protamine IgG antibody in the reactor's serum was measured using the technique of heterologous interpolation described previously [30,31].

Briefly, a serum pool containing 15400 ng/ml of anti-ragweed IgG antibody, measured using an elution technique [24], was used to generate a standard curve in a solid-phase radioimmunoassay measuring anti-ragweed IgG antibody [24,25]. Concurrently, multiple twofold dilutions of the protamine reactor's serum were analysed using the protamine-agarose solid phase. The standard dilution curve was fitted mathematically by a three-parameter spline function as previously described [32], and the amount of IgG antibody directed against protamine was interpolated from the ragweed standard curve.

Intradermal skin testing

Intradermal injections were performed using a technique described previously [33]. Briefly, approximately 0.02 ml was injected, in duplicate, on the volar aspect of the lower arm, raising a 3–4 mm weal. After 15 min, the site was inspected and the longest and mid-point orthogonal weal-and-flare diameters were measured and recorded. The sum of the longest and mid-point orthogonal diameters were divided by 2 and the means of the duplicates recorded. Protamine sulphate was diluted with sterile albumin saline with phenol (Hollister-Stier, Spokane, U.S.A.) immediately before use. Skin testing was also performed with histamine phosphate (0.275 mg/ml) (Center Labs, Fort Washington, New York, U.S.A.), sterile albumin saline diluent, and rabbit F(ab')₂ anti-human IgE antibody (1 µg/ml), which we have previously shown to cause *in-vivo* skin mast cell degranulation in all individuals tested to date [34].

In-vitro basophil histamine release

After informed consent, basophils were obtained from peripheral blood as previously described [35]. Briefly, 30 ml of blood were mixed with 6.25 ml of 6% dextran and 2.5 ml of 0.1 M EDTA and allowed to sediment by gravity for 90 min. The leucocyte-containing plasma layer was then removed and cells were sedimented by centrifugation (150 × *g* for 10 min at 4°C), washed twice in PAG buffer (25 mM PIPES, 110 mM sodium chloride, 5 mM potassium chloride, 0.003% HSA, and 0.1% D-glucose adjusted to pH 7.4 with 10 mM NaOH) (150 × *g* 8 min at 4°C), and resuspended in buffer containing 1 mM CaCl₂. Basophils (5 × 10⁴) were challenged with varying concentrations of protamine sulphate in a final volume of 100 µl. In half of the tubes deuterium oxide (D₂O, 26%) was added, because D₂O has been shown to enhance release by 'weak' stimuli [36]. After incubating for 45 min at 37°C the reactions were stopped by the addition of 0.9 ml cold PAG buffer. The tubes were then centrifuged (1000 × *g* for 2.5 min at

21°C) and aliquots of the cell-free supernatant were assessed for histamine content by the automated fluorometric assay of Siraganian [37]. The results were based on the mean of duplicate determinations and were expressed as percentage histamine release by dividing by total cellular histamine after subtracting for spontaneous release of unstimulated basophils. Total cellular histamine was obtained by lysing cells in 2% perchloric acid.

Results

Standardization of anti-protamine IgE and IgG antibody content in the protamine reactor's serum

There was no absorption of IgE antibody from the protamine reactor's serum after incubation with the agarose (sham) sorbent, while incubation with the protamine-agarose sorbent depleted IgE antibody binding to the protamine-agarose complex by 82% (Fig. 1). The supernatant after immunoabsorption with the agarose (sham) sorbent contained 135 ± 1.5 ng/ml (range) IgE while the supernatant after immunoabsorption with the protamine-agarose sorbent contained 122 ± 1.2 ng/ml IgE (*n* = 2, independent depletion experiments). Therefore, the protamine reactor's serum contained 16 ng/ml of IgE antibody directed against protamine which was 12% of the total IgE content.

Heterologous interpolation from a calibrated ragweed IgG antibody standard curve indicated that the highest serum titre from the protamine reactor had 21.7 ± 1.6 µg/ml (range) of IgG antibody directed against prota-

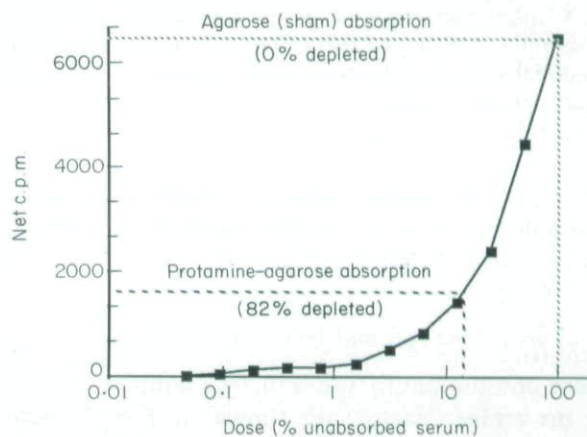


Fig. 1. Absorption of IgE antibodies from the protamine reactor's serum after incubation with the agarose (sham) sorbent and after incubation with the protamine-agarose sorbent. Interpolation from a standard curve (generated using unabsorbed serum from the protamine reactor) indicated that the protamine-agarose sorbent depleted IgE antibody to protamine by 82%.

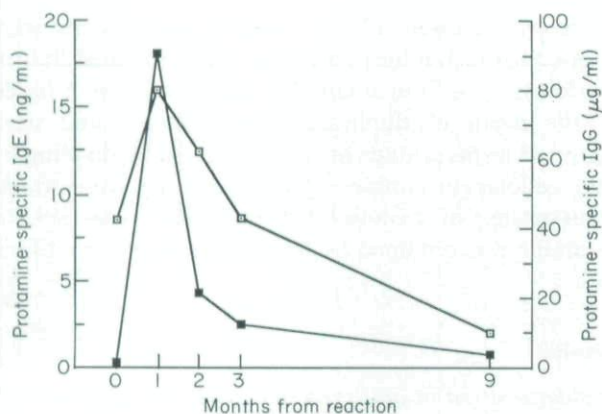


Fig. 2. Serial anti-protamine IgE (□) and IgG (■) antibody levels in the serum of the protamine reactor. At the time of the reaction, the patient's serum contained 8.5 ng/ml of anti-protamine IgE and 1.3 µg/ml of anti-protamine IgG antibody.

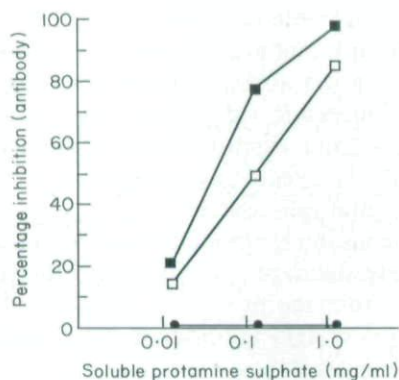


Fig. 3. Specific inhibition of anti-protamine IgE (□) and IgG (■) antibody binding to the protamine-agarose solid phase. Protamine did not inhibit binding of ragweed IgE antibody to a ragweed sorbent (●).

mine ($n=2$). Protamine and ragweed curves were parallel as reflected by an inter-dilutional coefficient of variation of only 12% (data not shown).

Serial protamine IgE and IgG antibody levels

Serum anti-protamine IgE and IgG antibody levels in the protamine reactor are shown in Fig. 2. Serum obtained the day of the reaction contained 8.5 ng/ml of anti-protamine IgE and 1.3 µg/ml anti-protamine IgG antibody. One month after his severe protracted reaction, both anti-protamine IgE and IgG antibody levels were increased (16 ng/ml (twofold rise) and 90.5 µg/ml (70-fold rise), respectively). This was followed by a subsequent decline in both anti-protamine IgE and IgG

antibody levels (Fig. 2). As noted above, the patient was switched from NPH insulin to lente insulin after his reaction.

Pre-incubation with increasing amounts of soluble protamine caused a dose-dependent inhibition of both anti-protamine IgE and IgG antibody binding to the protamine-agarose solid phase (Fig. 3). The specificity of the inhibition was confirmed by the inability of soluble protamine (up to 1.4 mg/ml) to inhibit the direct binding of ragweed-specific IgE antibody to a ragweed-agarose sorbent (mean inhibition = 0%; $n=4$).

Serial intracutaneous skin tests

Intradermal skin responses to protamine sulphate 1, 2, 3 and 9 months following the reaction were not significantly different in the protamine reactor compared to nine control subjects. The control subjects had no prior exposure to protamine, no risk factor for protamine antibody production, and no anti-protamine IgE antibody as measured by RAST (Table 1). All control subjects had a weal-and-flare response at 1000 µg/ml and four of nine had skin reactions at 100 µg/ml. Both histamine phosphate and F(ab')₂ anti-human IgE antibody produced weal-and-flare reactions in the protamine reactor.

Basophil histamine release to protamine sulphate

Washed leucocytes from the protamine reactor and an unexposed control were incubated with increasing concentrations of protamine (0.1–1000 µg/ml) 1 and 9 months after the protamine reaction. One month after the protamine reaction, when the reactor's serum contained 16 ng/ml of anti-protamine IgE antibody, the protamine reactor's basophils released 2.5% of total cellular histamine when incubated with 0.1 µg/ml of protamine sulphate. Increasing concentrations of protamine sulphate caused a slight increase in the percentage histamine released. Deuterium oxide (26%) co-incubated with protamine sulphate had a variable effect on the basophil histamine release. An unexposed control subject's basophils released no histamine until incubated with ≥ 100 µg/ml of protamine sulphate. Co-incubation with D₂O did not significantly affect histamine release (Fig. 4a). Nine months after the protamine reaction, when the reactor's serum contained 2 ng/ml of anti-protamine IgE antibody, the protamine reactor's basophils released 6% of total cellular histamine when incubated with 0.1 µg/ml of protamine sulphate. Increasing concentrations of protamine sulphate caused a slight, dose-dependent increase in the percentage histamine released. Co-incubation with D₂O enhanced the percent-

Table 1. Intradermal skin testing of protamine reactor and normal subjects

Skin-test reagent ($\mu\text{g/ml}$)	Protamine reactor (months after reaction)				Normal subjects								
	1	2	3	9	1	2	3	4	5	6	7	8	9
Protamine sulphate													
1	0	0	0	0	0	0	0	0	0	0	0	0	0
10	6/0	0	0	0	0	0	0	0	0	0	0	0	0
100	8.5/15	0	0	5/0	0	0	+/-	8/8	0	0/15	0/7	0/5	0
1000	9.5/36	7/10	10/19	5/0	10/14	7/23	0/18	10/14	0/22	8/32	9/26	7/15	8/15
Histamine phosphate (275)	12/46	10.5/35	11/32	8/23	13/30	12/50	8/24	11/42	12/38	12/45	10/50	12/48	9/25
F(ab') ₂ anti-human IgE (1)	n.d.	9/26	10.5/31	9/21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PBS-HSA	0	0	0	0	0	0	0	0	0	0	0	0	0

Skin tests recorded as weal/flare (mm).

PBS-HSA, phosphate-buffered saline with phenol and human serum albumin.

All normal subjects had negative *in-vitro* immunoassays for protamine IgE antibody.

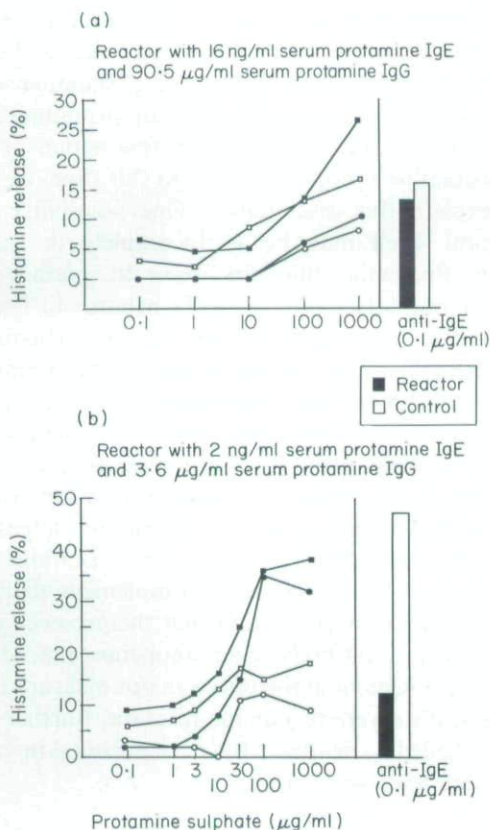


Fig. 4. Basophil histamine release to protamine and anti-IgE in the protamine reactor ($\square$, $\blacksquare$) and a non-exposed control ($\circ$, $\bullet$), (a) 1 and (b) 9 months after reaction. Net histamine release in buffer is shown as open symbols; net release in 26% D_2O is shown as closed symbols.

age histamine released. A second unexposed control subject's basophils released 3% of total cellular histamine when incubated with 0.1 $\mu\text{g/ml}$ of protamine sulphate. Only when incubated with ≥ 30 $\mu\text{g/ml}$ of protamine sulphate did the unexposed control's basophils increase the percentage histamine released. Co-incubation with D_2O enhanced the ability of protamine to release histamine from the unexposed control subjects' basophils only at concentrations of protamine sulphate ≥ 10 $\mu\text{g/ml}$ (Fig. 4b). Both the protamine reactor's and the unexposed control subject's basophils released histamine when incubated with anti-IgE (0.1 $\mu\text{g/ml}$) (Fig. 4).

Discussion

The mechanism(s) by which protamine causes its adverse reactions are not completely understood, although it appears likely that more than one mechanism may be involved. Protamine has been shown to complex with heparin and activate complement *in vitro* [13] and *in vivo* [14–16,18]. Studies have shown that diabetic patients receiving daily protamine-insulin injections have a 40–50-fold increased risk for acute reactions when given intravenous protamine [6,7] and we have previously shown that in this same high risk population the presence of anti-protamine IgE or IgG antibodies was a significant risk factor for acute protamine reactions [11]. These studies suggest that in protamine-insulin dependent diabetics, antibody-mediated mechanisms are involved in acute reactions to intravenous protamine.

Anti-protamine IgG antibody may cause protamine

reactions by activating complement and generating the anaphylatoxins C3a, C4a, and C5a [8–11]. The generation of anaphylatoxins and an increase in plasma thromboxane levels have been associated with pulmonary vasoconstriction which may occur following protamine administration [5,18,38]. Although usually mild and self-limited, acute pulmonary artery hypertension, when severe, may lead to right ventricular failure, diminished left ventricular output, and systemic hypotension. Anti-protamine IgE antibody fixed to mast cells and/or basophil via high affinity IgE receptors may cause mast cell and/or basophil mediator release after interaction with protamine. Furthermore, protamine may potentiate IgE antibody-dependent mediator release from human basophils [22].

The patient described in this report had been receiving neutral protamine Hagerdorn (NPH) insulin for 10 years before receiving intravenous protamine. He denied any adverse effects (induration, pruritus, rash, urticaria or systemic reactions) associated with his daily subcutaneous NPH insulin injections. Slow intravenous administration of protamine nevertheless provoked profound, life-threatening hypotension which required intensive therapy for almost 48 hr. Although atypical, such protracted hypotension has been reported after protamine administration in the past [39].

At the time of his protamine reaction, the patient's serum contained 8.5 ng/ml of anti-protamine IgE antibody and 1.3 µg/ml of anti-protamine IgG antibody. One month following his protracted protamine reaction, anti-protamine IgE antibody was increased twofold while anti-protamine IgG antibody increased 70-fold (Fig. 2). This boost in antibody levels is typical when patients are re-exposed to antigens to which they have previously been sensitized.

Skin testing with protamine was essentially negative in this patient since weal-and-flare responses were only observed at concentrations which produced non-specific skin reactions in normal controls. Yet, the reactor's skin mast cells were able to respond to IgE-dependent stimuli, as shown by the positive skin-test response to anti-IgE. It is theoretically possible that the profound reaction to protamine caused a prolonged state of antigen-specific desensitization. However, there is no evidence in the literature to support this, and we believe it to be highly unlikely. Although the literature contains reports of patients with protamine reactions who had positive protamine skin tests, two of these reports [2,9] do not provide information on the concentrations of protamine used for skin tests, and a third report [40] employed concentrations of protamine that we have shown cause false-positive, irritative reactions in the skin. Lakin *et al.* [8] reported a protamine reactor with a positive skin test

to protamine at 0.001 µg/ml, which should be a non-irritative concentration. A recent study found that results of protamine skin testing were not useful in predicting subsequent protamine reactions [41].

In-vitro basophil histamine release studies with protamine were inconclusive. Both 1 and 9 months after his protamine reaction, the reactor's basophils released more histamine when incubated with protamine than did basophils from unexposed controls (Fig. 4), although this difference was not great. Protamine, at concentration ≥ 30 µg/ml, produced histamine release from basophils from unexposed controls, which suggests that at these higher concentrations (not reached in *in-vivo* administration), histamine release is caused by a non-immunological mechanism.

The presence of anti-protamine IgE antibody in this patient's serum at the time of intravenous protamine exposure, and the boost in anti-protamine IgE levels 1 month following the exposure, along with the increased risk of protamine reactions in protamine-insulin dependent diabetic patients who have anti-protamine IgE antibodies [11] supports the concept that protamine is both immunogenic and antigenic. However, the negative protamine skin test results, along with the equivocal *in-vitro* basophil-histamine release to protamine and the fact that subcutaneous injections of protamine-insulin produced no adverse reactions in this patient, suggests that protamine is non-antigenic. At this time, we cannot fully explain this discrepancy. One possibility is that protamine itself may be an incomplete or univalent antigen. Protamine may first have to interact with a tissue macromolecule or possibly heparin to become a complete or multivalent antigen capable of eliciting IgE-dependent mediator release. Experiments in sheep and pigs have shown that administration of intravenous protamine alone does not produce haemodynamic changes whereas protamine administered after heparin anti-coagulation induces pulmonary vasoconstriction [21,42–44]. Another possibility is that the increased risk for acute protamine reactions in protamine-insulin dependent diabetics is due to complement fixing anti-protamine IgG antibody, and that the presence of anti-protamine IgE antibody is an epiphenomena. Unfortunately, complement activation was not measured during this patient's severe protamine reaction. Further studies will be needed to delineate the mechanism(s) involved in protamine reactions better.

Acknowledgment

Supported in part by grant (AI 20136) from the National Institutes of Health and by the United States Pharmacopeial Convention.

References

- Sharath MD, Metzger WJ, Richerson HB, *et al.* Protamine-induced fatal anaphylaxis. *J Thor Cardiovasc Surg* 1985; 90:86-90.
- Doolan L, McKenzie I, Krafchek J, Parson B, Buxton B. Protamine sulphate hypersensitivity. *Anaesth Intn Care* 1981; 9:147.
- Chung F, Miles J. Cardiac arrest following protamine administration. *Can Anaesth Soc J* 1984; 31:314-18.
- Vontz FK, Puestow EC, Cahill DJ Jr. Anaphylactic shock following protamine administration. *Am Surgeon* 1982; 48:549-51.
- Lowenstein E, Lynch K, Robinson DR, Fitzgibbon CM, Zapol W. Incidence, severity and causation of adverse cardiopulmonary response to protamine reversal of heparin anticoagulation in man. *Am Rev Respir Dis* 1988; 137:245(abstr).
- Stewart WJ, McSweeney SM, Kellett MA, Faxon DP, Ryan TJ. Increased risk of severe protamine reactions in NPH insulin-dependent diabetics undergoing cardiac catheterization. *Circulation* 1984; 70:788-92.
- Gottschlich GM, Gravlee GP, Georgitis JW. Adverse reactions to protamine sulfate during cardiac surgery in diabetic and non-diabetic patients. *Ann Allergy* 1988; 61:277-81.
- Lakin JD, Blocker TJ, Strong DM, Yocum MW. Anaphylaxis to protamine sulfate mediated by a complement-dependent IgG antibody. *J Allergy Clin Immunol* 1977; 61:102-7.
- Grant JA, Cooper JR, Albyn KC, Buhner D, Farnam J, Yunginger JW. Anaphylactic reactions to protamine in insulin-dependent diabetics after cardiovascular procedures. *J Allergy Clin Immunol* 1984; 73:180 (abstr).
- Gottschlich GM, Georgitis JW. Protamine-specific IgE, IgG, and IgG subclass antibodies in protamine anaphylaxis. *J Allergy Clin Immunol* 1988; 81:238 (abstr).
- Weiss ME, Nyhan D, Zhikang P, *et al.* Association of protamine IgE and IgG antibodies with life-threatening reactions to intravenous protamine. *N Engl J Med* 1989; 320:886-92.
- Keller R. Interrelations between different types of cells. *Int Arch Allergy* 1968; 34:139-44.
- Rent R, Ertel N, Eisenstein R, Gewurz H. Complement activation by interaction of polyanions and polycations. I. Heparin-protamine induced consumption of complement. *J Immunol* 1975; 114:120-4.
- Best N, Teisner B, Grudzinskas JG, Fisher MM. Classical pathway activation during an adverse response to protamine sulphate. *Br J Anaesth* 1983; 55:1149-53.
- Best N, Sinosich MJ, Teisner B, Grudzinskas JG, Fisher MM. Complement activation during cardiopulmonary bypass by heparin-protamine interaction. *Br J Anaesth* 1984; 56:339-43.
- Kirklin JK, Chenoweth DE, Naftel DC, *et al.* Effects of protamine administration after cardiopulmonary bypass on complement, blood elements, and the hemodynamic state. *Ann Thor Surg* 1986; 41:193-9.
- Skidgel RA, Tan F, Jackman H, Zsigmond EK, Erdos EG. Protamine inhibits plasma carboxypeptidase N (CPN), the inactivator of anaphylatoxins and kinins. *Fed Proc* 1988; 2:a1382 (abstr).
- Morel DR, Zapol WM, Thomas SJ, *et al.* C5a and thromboxane generation with pulmonary vaso- and broncho-constriction during protamine reversal of heparin. *Anesthesiology* 1987;66:597-604.
- Hobbbahn J, Conzen PF, Zenker B, Goetz AE, Peter K, Brendel W. Beneficial effect of cyclooxygenase inhibition on adverse hemodynamic responses after protamine. *Anesth Analg* 1988; 67:253-60.
- Degges RD, Foster ME, Dang AQ, Read RC. Pulmonary hypertensive effect of heparin and protamine interaction: evidence for thromboxane B2 release from the lung. *Am J Surg* 1987; 154:696-9.
- Morel DR, Lowenstein E, Nguyenduy T, *et al.* Acute pulmonary vasoconstriction and thromboxane release during protamine reversal of heparin anticoagulation in awake sheep. Evidence for the role of reactive oxygen metabolites following nonimmunological complement activation. *Circulation Res* 1988; 62:905-15.
- Tobin MC, Karns BK, Anselmino LM, Thomas LL. Potentiation of human basophil histamine release by protamine: a new role for a polycation recognition site. *Mol Immunol* 1986; 23:245-53.
- Adkinson NF, Jr. Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody. In: Rose NF, Friedman H, eds. *Manual of clinical immunology*. Washington, DC: American Society for Microbiology, 1986:664-74.
- Hamilton RG, Adkinson NF Jr. Quantitation of antigen-specific IgG in human serum standardization by a staph A solid phase radioimmunoassay elution technique. *J Immunol* 1980; 124:2966-71.
- Hamilton RG, Sobotka AK, Adkinson NF Jr. Solid phase radioimmunoassay for quantitation of antigen-specific IgG in human sera with ¹²⁵I-protein A from *Staphylococcus aureus*. *J Immunol* 1979; 122:1073-9.
- Hamilton RG, Rendell M, Adkinson NF, Jr. Serological analysis of human IgG and IgG and IgE anti-insulin antibodies by solid-phase radioimmunoassays. *J Lab Clin Med* 1980; 96:1022-36.
- Hamilton RG, Adkinson NF, Jr. Naturally occurring carbohydrate antibodies: interference in solid-phase immunoassays. *J Immunol Methods* 1985; 77:95-108.
- Gleich GJ, Jacob GL. Immunoglobulin E antibodies to pollen allergens account for high percentages of total immunoglobulin E protein. *Science* 1975; 190:1106-8.
- Gleich GJ, Jacob GL, Yunginger JW, Henderson LL. Measurement of the absolute levels of IgE antibodies in patients with ragweed hay fever. *J Allergy Clin Immunol* 1977; 60:188-98.
- Hamilton RG, Kagey-Sobotka A. Immunoassay quantitation of allergen-specific IgG 'blocking' antibody. *J Clin Immunoassay* 1983; 6:160-71.
- Hamilton RG, Adkinson NF, Jr. Quantitative aspects of solid phase immunoassays. In: Kemeny DM, Challacombe

- SJ, eds. ELISA and other solid phase immunoassays. Chichester: John Wiley & Sons, 1988:57-84.
- 32 Rogers FD, Adams JA. Space curves. In: Rogers FD, Adams JA, eds. Mathematical elements for computer graphics. New York: McGraw-Hill, 1976:133-8.
- 33 Norman PS. Skin testing. In: Rose NR, Friedman H, Fahey JL, eds. Manual of clinical laboratory immunology. Washington DC: American Society for Microbiology, 1986:660-3.
- 34 Weiss ME, Trent P, Fisher R, Norman PS, Waterbury WE, Adkinson NF, Jr. Rabbit F(ab')₂ anti-human IgE is a 'universal' skin test reagent in the evaluation of skin mast cell degranulation in vivo. *J Allergy Clin Immunol* 1989; 83:1040-8.
- 35 May CD, Lyman M, Alberto R, Cheng J. Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. *J Allergy* 1970; 46:12-20.
- 36 Dembo M, Goldstein B, Sobotka AK, Lichtenstein LM. Histamine release due to bivalent penicilloyl haptens: control by the basophil plasma membrane. *J Immunol* 1978; 121:354-8.
- 37 Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975; 7:283-9.
- 38 McIntyre RW, Flezzani P, Knopes KD, Reves JG, Watkins WD. Pulmonary hypertension and prostaglandins after protamine. *Am J Cardiol* 1986; 58:857-8.
- 39 Jackson DR. Sustained hypotension secondary to protamine sulfate. *Angiol* 1970; 21:295-8.
- 40 Knappe JTA, Schuller JL, De Haan P, De Jong AP, Bovill JG. An anaphylactic reaction to protamine in a patient allergic to fish. *Anesthesiology* 1981; 55:324-5.
- 41 Vegh AB, Gellhaus MA, Benson PM, Hottel RA, Weiler JM. Protamine sulfate skin testing does not predict immediate adverse systemic reactions following protamine administration. *J Allergy Clin Immunol* 1989; 18:256 (abstr).
- 42 Fiser WP, Fewell JE, Hill DE, Barnes RW, Read RC. Cardiovascular effects of protamine sulfate are dependent on the presence and type of circulating heparin. *J Thorac Cardiovasc Surg* 1985; 89:63-70.
- 43 Radegran K, Taylor GA, Olsson P. Mode of action of protamine in regard to its circulatory and respiratory side effects. *Eur Surg Res* 1971; 3:139-48.
- 44 Stefaniszyn HJ, Novick RJ, Salerno TA. Toward a better understanding of the hemodynamic effects of protamine and heparin interaction. *J Thorac Cardiovasc Surg* 1984; 87:678-86.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.