

Anaphylaxis to protamine sulfate mediated by a complement-dependent IgG antibody*

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Anaphylactoid reactions to protamine sulfate have been attributed to its capacity for nonimmunologic mast cell degranulation and/or complement consumption. In the current study, evidence is presented for the occurrence of an immunologic anaphylactic reaction mediated by a complement-dependent IgG skin-sensitizing antibody. A retrospective study of blood component donors given protamine for heparin neutralization revealed that prior exposure to protamine is associated with increased risk of adverse reaction to the drug.

Protamine is a strongly polycationic polypeptide which has been employed with increasing frequency for heparin neutralization in cardiopulmonary bypass procedures and in blood component donation, by cell separator techniques.¹⁻² With increased usage increased numbers of adverse reactions to this biological have been encountered, frequently including hypotensive episodes and/or generalized urticaria.³ Protamine, in both dog and man, causes a decrease in systemic vascular resistance in conjunction with a marked fall in cardiac output and concomitant progressive increase in pulmonary artery pressure. Presumably, these vascular changes are mediated at least in part by release of vasoactive platelet substances.³⁻⁴ Further, protamine has been reported to

degranulate mast cells in vitro causing histamine release.⁵ Also, protamine in common with other polyanions and polycations is anticomplementary. It appears that complement activation is mediated via the classical pathway through which C3a, C5a, anaphylatoxin generation may occur.⁶⁻⁷ Thus, it has been commonly assumed that hypotensive and urticarial responses to protamine administration are nonimmunologic.⁸⁻⁹ However, our laboratory has recently accumulated evidence suggesting that at least in some instances a humoral immunologic mechanism may be operative.¹⁰

CASE HISTORY

Patient J. K. is a 29-yr-old white man in good general health. Five months prior to his reaction he had donated blood components on an AMINCO IBM cell separator, being anticoagulated with heparin and subsequently given intravenous protamine without difficulty. Prior to a second donation, the patient received 2,500 U of heparin intravenously. He was placed on the separator and given an additional 4,000 U of heparin by constant infusion over the next 4 hr. At the termination of the procedure, the patient received 6.5 mg protamine sulfate intravenously over 30 sec. Within one-half minute the patient complained of dyspnea. Within 30 sec generalized urticaria and audible wheezing were observed, immediately followed by syncope, acrocyanosis, and hypotension. Diphenhydramine 50 mg was administered intravenously 4 min after the protamine, in conjunction with oxygen therapy. The patient's symptoms resolved several minutes thereafter. Approximately 12 months after this episode, the patient was referred to our laboratory for study. At that time, serum concentrations of C3, C4, IgG, IgA, IgM, and IgD were within normal limits. IgE concentration was 37 IU/ml. Circulating tetanus antitoxin was at a concentration of 1.0 tetanus antitoxin

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units/ml. Lymphocyte transformation studies to streptokinase-streptodornase, phytohemagglutinin, and tetanus toxoid were normal, relative to a panel of 5 healthy young adult white men. Delayed skin testing was positive to purified protein derivative S. Past medical history and family history were negative for atopic disease.

MATERIALS AND METHODS

Skin testing

Direct prick skin testing was performed on the patient by standard methods¹¹ using dilutions of USP sterile protamine sulfate (Eli Lilly and Company, Lot No. 8EF68A) in sterile phosphate-buffered saline, pH 7.4 (PBS). Prausnitz-Küstner (P-K)-type passive cutaneous transfer reactions were performed using normal adult rhesus monkeys as described previously.¹² An antigen dose of 0.1 mcg in 0.1 ml PBS was used to challenge the P-K sites at 4 and 48 hr. A nonirritating antigen dosage was determined by dilution titration of protamine sulfate alone in rhesus monkeys injected intravenously with Evans blue dye, 2.5 ml.

Removal of IgG, IgA, IgM, and IgE antibody activity

The IgG, IgA, or IgM fractions of patient's serum were removed by sequential direct precipitation with high-titer rabbit antihuman γ -, α - and μ - chain antisera, respectively.¹³⁻¹⁴ Completeness of immunoglobulin removal was monitored by radial diffusion for IgG, IgA, or IgM by the Mancini technique (Behring Diagnostics).¹⁵ IgE was removed from serum by a double-antibody coprecipitation technique using burro antihuman ϵ -chain antiserum, previously demonstrated to be monospecific for IgE,¹⁴ followed by rabbit antiburro IgG antiserum. Also, samples of patient's serum were heated to 56° C for 30 min to remove IgE-mediated antibody activity.¹²

Reverse passive cutaneous anaphylaxis (RPCA).

This technique¹⁶ was performed in quadruplicate using guinea pigs sensitized by intravenous injection of 12 ml patient's serum or normal human serum. Seventy-two hours later, 1 ml Evans blue dye was injected intravenously and protamine sulfate at various concentrations in PBS was injected intradermally. The animals were sacrificed, skinned, and results read at 20 min.

Effect of de complementation on P-K reactivity

Complement dependence of serum skin-sensitizing activity was assayed by P-K testing of a rhesus monkey before and after de complementation. De complementation was performed by injection of freshly reconstituted cobra venom factor (CVF) (Cordis Laboratories), 200 anticomplementary units, intraperitoneally, into a 9.1 kg rhesus monkey every 12 hr for 60 hr or 5 doses.¹⁷ Twelve hours after the last injection and 4 hr after intradermal injection of serum, P-K sites were challenged with protamine and read at 20 min. The effectiveness of the de complementation regime was

TABLE I. Direct prick skin testing of Patient J. K. and normal adult males with protamine sulfate

Protamine (mcg/ml)	Patient J. K.	Normal controls				
		1	2	3	4	5
1,000		—	2+	2+	±	1+
100	4+	—	±	—	—	±
10	4+	—	—	—	—	—
1	4+	—	—	—	—	—
0.1	3+	—	—	—	—	—
0.01	3+	—	—	—	—	—
0.001	2+	—	—	—	—	—
0.0001	±	—	—	—	—	—
PBS*	—	—	—	—	—	—

*Sterile phosphate-buffered saline, pH 7.4.

monitored by determination of C3 serum concentrations by radial immunodiffusion (Behring Diagnostics) before and after administration of CVF. C3 concentrations were measured as cross-reacting units/dl, using a single lot of anti-human C3 for radial immunodiffusion,¹² and the percent reduction of C3 concentration calculated. As a positive control, a serum containing IgE antiragweed antibody was concomitantly titrated by P-K in the de complemented rhesus monkey. IgE antiragweed serum dilutions were injected intradermally 48 hr prior to challenge.

Retrospective analysis of adverse reactions associated with protamine administration

A total of 44 consecutive blood component donations by AMINCO IBM cell separator made by a population of 22 donors following the protocol outlined in the case history were retrospectively analyzed. Any urticarial, bronchospastic, and/or hypotensive reactions noted in their medical records were correlated with the number of prior donations an individual had made. Chi-square analysis was used to compare frequency of observed reactions among the various groups.

RESULTS

On direct prick testing of the patient with protamine sulfate, whealing could be elicited by solutions containing as little as a nanogram of protamine per milliliter. In contrast, the threshold for presumably irritative skin reactivity to protamine in 5 normal young white men was from 1 to 10 million times greater at approximately 1 mg/ml (Table I). P-K type passive cutaneous transfer reactions in a panel of 2 rhesus monkeys were negative when sites prepared with serum dilutions were challenged with protamine at 48 hr. However, when challenge was performed at 4 hr following intradermal injection of serum, skin-sensitizing activity was repeatedly detected in titers of 1:64 to 1:128. This skin-sensitizing activity was unaffected by heating the serum to 56° C for 30 min or

TABLE II. P-K transfer of antiprotamine skin-sensitizing activity to rhesus monkey*

Dilution	4 hr						48 hr		
	Patient J. K. serum						Normal human serum	Patient J. K. serum untreated	Normal human serum
	Untreated	56°, 30 min	-IgE	-IgG	-IgA	-IgM			
1:2	+	+					-	-	-
1:4	+	+	+	-	+	+	-	-	-
1:8	+	+	+	-	+	+	-	-	-
1:16	+	+	+	-	+	+	-	-	-
1:32	+	+	+	-	+	+	-	-	-
1:64	+	+	+	-	+	+	-	-	-
1:128	±	-	±	-	-	+	-	-	-
1:256	-	-	-	-	-	-	-	-	-

*P-K sites were challenged with 0.1 mcg protamine sulfate in 0.1 ml phosphate-buffered normal saline.

TABLE III. RPCA activity of Patient J. K. serum in guinea pig*

Protamine (mcg)	Patient J. K. serum	Normal human serum
75	+	±
40	+	-
20	+	-
10	+	-
5	+	-
0	-	-

*Guinea pigs were sensitized by injection of 12 ml serum and were challenged intradermally with protamine sulfate 72 hr later.

by removal of IgE by double-antibody coprecipitation. However, removal of serum IgG abolished 4-hr skin-sensitizing activity. Removal of IgM or IgA was without effect (Table II). In the P-K challenges a dosage of 0.1 mcg protamine sulfate in 0.1 ml of PBS was used. The amount was nonirritating when injected intradermally into monkey skin. The nature of the skin-sensitizing activity in the patient's serum was further examined by RPCA in guinea pigs (Table III). Seventy-two hours after intravenous sensitization with 12 ml of serum, those animals which received the patient's serum had positive RPCA reactions to challenge with protamine sulfate in amounts ranging from 5 to 75 mcg. In contrast, those animals which had received normal human serum 72 hr earlier had no RPCA activity on challenge (Table III). The degree of complement dependence, of the observed 4-hr P-K reaction of patient's serum and protamine sulfate was assessed by decomplexation of the recipient rhesus monkey with C566. Reduction of C3 in the monkey to 14% of predecomplexation level was noted. Complement depletion abolished the ability of patient's serum to produce positive P-K reactions on challenge with protamine. A 48-hr ragweed IgE an-

tiragweed P-K was not influenced by the decomplexation procedure (Table IV).

The incidence of adverse reaction to protamine administration as a function of prior administration was studied by retrospective examination. The records of 44 consecutive donations on an AMINCO IBM cell separator were reviewed. Total-body heparinization was followed by protamine administration in each instance. Out of 22 initial protamine injections, no adverse reactions were observed. However, out of 11 donors receiving protamine a second time, 4 urticarial episodes with or without bronchospasm or hypotension were noted ($p < 0.005$). The average time from first protamine exposure to occurrence of adverse reaction was 32.8 days with a range of 12 to 120 days.

DISCUSSION

Urticarial and anaphylactoid reactions following protamine administration have been observed for over thirty years. In initial clinical reports an atopic mechanism was not thought to be operative as direct skin testing and tests for precipitating antibody were negative.^{8, 9} As the vasoactive properties of protamine were further elucidated, a number of nonimmunologic reactions capable of producing anaphylactoid symptoms have been documented. In both dog models and in man intravenous injection of protamine is rapidly followed by a decrease in systemic vascular resistance and concurrent fall in cardiac output producing peripheral hypotension. Additionally, a gradual sustained increase in pulmonary vascular resistance occurs.^{3, 18, 19} These phenomena were attributed to peripheral pooling of the vascular compartment.¹⁹ These properties of protamine may be explained by several in vitro characteristics of the agent. Many polycationic polypeptides including protamine are active in releasing histamine from rat, hamster, and

TABLE IV. The effect of C3 depletion of the recipient rhesus monkey on P-K activity

Dilution of serum	Prior to C3 depletion		Following C3 depletion	
	Patient J. K. serum*	IgE-antiragweed serum†	Patient J. K. serum*	IgE-antiragweed serum†
1:2	+	+	—	+
1:4	+	+	—	+
1:8	+	+	—	+
1:16	+	+	—	+
1:32	+	+	—	+
1:64	+	+	—	+
1:128	±	+	—	+
1:256	±	+	—	—
PBS	—	—	—	—

*The site of intradermal serum dilution injection was challenged 4 hr later by injection of protamine.

†The site of intradermal serum dilution injection was challenged 48 hr later by injection of aqueous ragweed extract.

TABLE V. Incidence of urticarial, bronchospastic, and/or hypotensive reactions to protamine among 44 administrations of the drug to 22 blood component donors

Number of exposures	1		2*		3		4		5	
Adverse reactions	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Number of donors	0	22	4	7	1	4	0	3	0	2
Percent having adverse reactions	0%		36%		20%		0%		0%	

*p < 0.005 between 1 exposure and 2 exposure groups.

rabbit mast cells.⁵ This process, in a clinical setting, could induce a number of anaphylactoid symptoms. Alternately, vigorous complement consumption can be induced by protamine in sera rich in C-reactive protein (CRP). Protamine apparently acts as a substrate of CRP, CRP in turn being a potent activator of the complement system at the level of C1.⁶ This in turn would lead to C3a, C5a, anaphylatoxin generation.²⁰ Furthermore, heparin and protamine in fresh human serum, in amounts much lower than those required for complement depletion by either agent alone, have been shown to induce virtually complete depletion of total hemolytic complement via the classical pathway by interaction with the first component of complement.⁷ Therefore, it may be surmised that a number of the anaphylactoid episodes observed subsequent to protamine administration may well be secondary to nonimmunologic mast cell degranulation and/or complement consumption mediated by protamine or protamine-heparin complexes.

However, the studies presented in this communication are consistent with an immunologic mechanism operative in the genesis of anaphylaxis in our patient. Direct prick skin testing disclosed an unusual degree of dermal sensitivity to the whealing effect of protamine. In comparison to normal control patients, a definitely positive immediate reaction to protamine

could be produced with test solutions at a one million-to ten million-fold lesser concentration of the agent (Table I). This skin-sensitizing activity could be demonstrated in rhesus monkey skin prepared by intradermal injection of dilutions of patient's serum when challenged at 4 hr but not at 48 hr after intradermal injection of serum dilutions. This, coupled with the observation that heating patient's serum to 56° C for 30 min did not affect its sensitizing activity (Table II), suggested that IgG antibody might be involved. Subsequently, removal of IgG from the serum was found to abolish 4-hr P-K skin-sensitizing activity, although removal of IgE, IgA, or IgM did not appear to appreciably affect P-K titers. The absence of reaction when fresh normal human serum or normal saline were challenged with protamine would also be consistent with an immunologic mechanism causing the observed reactions. Furthermore, if nonimmunologic C1 activation, directly or through CRP, were to be occurring, removal of IgG would not be expected to affect the reaction. A second line of evidence, consistent with an IgG-mediated skin-sensitizing antibody specific for protamine being present in our patient, was obtained in passive cutaneous anaphylaxis (PCA) studies in guinea pigs. Human IgG but not IgE has been shown to be cytotoxic under the experimental conditions of our

study.^{13, 16} As seen in Table III, a positive intradermal reaction could be elicited at 72 hr after sensitization with J. K.'s serum using amounts of protamine nonirritating to guinea pig skin.

A number of investigators have presented evidence suggesting the presence of IgG antibody as an additional mediator of reaginic activity in man,^{21, 24} although it is generally accepted that the majority of antibodies responsible for Type I hypersensitivity reactions in man belong to the IgE class.²⁵ These IgG reagins presumably act through mast cell binding, causing degranulation and mediator release in analogy to IgE-mediated Type I reactions and independent of the complement system.²⁴ However, it would appear that the IgG skin-sensitizing antibody in the serum of our patient caused local anaphylaxis through a complement-dependent process. As seen in Table IV, when the serum was tested for 4-hr P-K activity in a rhesus monkey depleted of complement by repeated injection of C56, the reaginic activity was abolished. In contrast, a control of P-K system consisting of serum containing IgE antiragweed reagin challenged with aqueous extract of whole ragweed pollen was unaffected by the de complementation procedure. Although the pathway by which complement consumption occurred is not known from the current data, either classic or alternate pathway would be expected to generate C3a, C5a anaphylatoxin, vasoactive peptides capable of mediating the observed clinical and intradermal reactions.^{20, 26, 27} With the evidence that one patient had experienced an anaphylactic reaction to protamine, associated with a complement-dependent IgG skin-sensitizing antibody, a retrospective survey of adverse reactions to protamine was undertaken. A total of 22 blood component donors by IBM-AMINCO cell separator were studied. This population performed a total of 44 separate donations with systemic heparinization followed by intravenous administration of from 5 to 50 mg protamine sulfate. As presented in Table V, none of the 22 donors had difficulty on their initial exposure to protamine. However, of the 11 donors exposed to the agent a second time, 4 (36%) developed immediate anaphylactoid symptoms ($p < 0.005$). The mechanism or mechanisms involved in these donors' reactions are not known. However, it is concluded from these data that prior exposure to protamine does correlate with increased risk of drug reaction.

AUTHORS' NOTE ADDED IN PROOF

Nordström, Fletcher, and Pavek have recently reported a clinically similar protamine-induced anaphylactoid reaction occurring in a 6-yr-old undergoing cardiopulmonary bypass procedures (Acta Anaesth. Scand., 1978; in press.)

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