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ASSOCIATION OF PROTAMINE IgE AND IgG ANTIBODIES WITH LIFE-THREATENING REACTIONS TO INTRAVENOUS PROTAMINE

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Abstract Life-threatening reactions to intravenous protamine, administered to reverse heparin anticoagulation, have been reported with increasing frequency as a consequence of the escalating use of cardiac catheterization and coronary bypass surgery. Retrospective studies have shown that such reactions are more common in diabetic patients receiving daily subcutaneous injections of protamine-insulin preparations. To determine whether anti-protamine IgE or IgG antibodies might explain the increased risk for protamine reactions among patients with protamine-insulin-dependent diabetes, we conducted a case-control study of 27 patients (diabetic and nondiabetic) who had acute reactions to intravenous protamine and 43 diabetic patients who tolerated protamine without a reaction during diagnostic or surgical procedures. Cases and controls were grouped according to previous exposure to protamine-insulin preparations.

MEDICINAL uses of protamine (derived mainly from salmon milt)¹ include the reversal of heparin anticoagulation used during vascular surgery, cardiac catheterization, dialysis, and phoresis and the retardation of insulin absorption (as in isophane, protamine zinc, and human insulin). As cardiac catheterization and coronary-artery bypass surgery have been

in diabetic patients who had received protamine-insulin injections, the presence of serum anti-protamine IgE antibody was a significant risk factor for acute protamine reactions (relative risk, 95; $P = 1.0 \times 10^{-5}$), as was anti-protamine IgG (relative risk, 38; $P = 1.2 \times 10^{-5}$). No patients without previous exposure to protamine-insulin injections had serum protamine IgE antibodies. In this group, anti-protamine IgG antibody was a risk factor for protamine reactions (relative risk, 25; $P = 0.0062$).

We conclude that in protamine-insulin-dependent diabetes, the increased risk of serious reactions when intravenous protamine was given appeared to be caused largely by antibody-mediated mechanisms. In nondiabetic subjects, the presence of protamine IgG was significantly associated with an increased risk of acute protamine reactions, although many nondiabetic subjects who had reactions had no IgG antibodies. (*N Engl J Med* 1989; 320:886-92.)

performed with increasing frequency, the use of intravenous protamine to reverse heparin anticoagulation has increased proportionately. This has resulted in numerous reports of adverse reactions associated with the intravenous administration of protamine.²⁻⁶

Adverse reactions to protamine include rash, urticaria, bronchospasm, elevated pulmonary-artery pressure, and hypotension leading at times to cardiovascular collapse and death.² Diabetic patients receiving daily subcutaneous injections of insulin containing protamine appear to have a 40-fold to 50-fold increase in the risk of life-threatening reactions when given protamine intravenously.^{7,8} The causes of such adverse reactions are incompletely understood; however, the pattern of daily subcutaneous injections of a foreign protein such as protamine in diabetic patients, followed by anaphylactic reactions after the intrave-

nous administration of approximately 1000-fold larger doses of protamine, suggests that humorally mediated immune responses may play a part in these reactions. A few studies⁹⁻¹¹ involving small numbers of patients without suitable controls have reported anti-protamine IgG antibodies in patients with protamine-insulin-dependent diabetes who had reactions to intravenously administered protamine. Little is known about the IgE antibody responses to protamine. To determine whether anti-protamine IgE or IgG antibodies could explain the increased risk of protamine reactions among patients with protamine-insulin-dependent diabetes, we carried out a case-control study.

METHODS

Subjects

Case subjects were patients, diabetic and nondiabetic, who had had adverse reactions to intravenous protamine, defined as bronchospasm (an increase in the peak airway pressure of ≥ 20 mm Hg or wheezing), hypotension (a fall in the mean systemic blood pressure of ≥ 15 mm Hg), or an elevation of the mean pulmonary-artery pressure (an increase of ≥ 7 mm Hg) within 10 minutes after the intravenous administration of protamine in the absence of other causes. Of the 27 patients, reactions occurred in 21 during open-heart surgery, in 3 during vascular procedures, and in 2 during cardiac catheterization. One patient had reactions during cardiac catheterization and open-heart surgery. Case patients were grouped according to their exposure to subcutaneous injections of protamine and insulin: Group D had not received subcutaneous injections; Group E had received such injections. Group D consisted of 13 nondiabetic patients and 1 diabetic patient who had been treated only with oral hypoglycemic agents. All patients in Group E were diabetics who had had protamine-insulin injections.

The control group consisted of 43 consecutive diabetic patients who tolerated intravenous protamine after coronary-artery bypass surgery with no adverse reactions. The controls were also grouped according to their exposure to subcutaneous protamine-insulin injections: Group B had never received such injections, and Group C had received subcutaneous injections. Serum was obtained from the controls within 24 hours of the intravenous administration of protamine. Whenever possible, serum samples from the case patients were obtained at the time of the protamine reaction. Of the 27 patients who had reacted to protamine, serum samples were obtained from 20 within 24 hours, from 4 within 10 days, and from 3 more than 10 days after the reaction.

Control serum samples were also obtained from 13 nondiabetic subjects (including 3 atopic patients with total serum IgE levels above 1000 ng per milliliter) who had never been exposed to protamine in any form (Group A).

The study was approved by the institutional review boards of the institutions involved.

Protamine IgE Antibody Assay

Protamine sulfate (Eli Lilly, Indianapolis) was bound to Sepharose CL-4B beads activated with cyanogen bromide (Pharmacia, Piscataway, N.J.) as described elsewhere.¹² The level of IgE antibody to protamine was measured with the use of a standard agarose-based radioallergosorbent test.¹³ The inhibition of the binding of IgE antibody to the protamine-agarose complex was evaluated by a two-hour preincubation of serum samples with soluble protamine sulfate (1.4 mg per milliliter).

Protamine IgG Antibody Assay

The absolute quantity of specific anti-protamine IgG antibody in serum from a patient who had reacted to protamine was determined with the use of heterologous interpolation.^{13,14} This reference serum was then used to generate a standard curve in an indirect enzyme-linked immunosorbent assay (ELISA) to measure specific IgG anti-

protamine antibody in unknown serum samples (i.e., samples whose antibody status was unknown). Polystyrene microtiter plates (PGC Scientific Laboratories, Gaithersburg, Md.) were coated with protamine sulfate (100 ng per well) in 100 μ l of 0.05 M carbonate buffer (pH 9.6), incubated overnight at 4°C, and then washed three times with ELISA buffer (pH 7.4) consisting of 0.01 M sodium phosphate, 0.15 M sodium chloride, 0.05 percent Tween 20, and 0.2 percent bovine serum albumin.

One hundred microliters of a 1:100 or greater dilution of either the standard reference serum or unknown serum samples was added to antigen-coated wells. All dilutions above 1:100 were made in normal serum diluted 1:100 in ELISA buffer. After an overnight incubation at 4°C, the plates were washed three times with ELISA buffer and 100 μ l of mouse monoclonal anti-human IgG antibody (clone HP6064) (Unipath, Birmingham, U.K.) diluted 1:10,000 in ELISA buffer was added to each well. After a four-hour incubation at room temperature, the plates were washed three times with ELISA buffer, and 100 μ l of affinity-purified goat anti-mouse IgG conjugated with alkaline phosphatase (Tago, Burlingame, Calif.) diluted 1:8000 was added to each well and incubated for two hours at 37°C. The plates were washed three times with ELISA buffer, 100 μ l of *p*-nitrophenylphosphate¹⁵ was added to each well, and the plates were incubated overnight at 4°C. The reaction was stopped by the addition of 100 μ l of 1 M sodium hydroxide to each well, and the optical density was read at 410 nm on an automated MR 700 microplate reader (Dynatech Laboratories, Alexandria, Va.). The precision (the mean for 18 patients with positive serum samples) was 2.4 percent (coefficient of variation), and the sensitivity of the assay was 50 ng per milliliter for undiluted serum samples.

The inhibition of IgG antibody binding to protamine-coated microtiter wells was evaluated by the preincubation of serum samples with 100 μ l of soluble protamine sulfate (5 mg per milliliter) for two hours before they were added to the microtiter plate. In all immunoassays, the serum samples were evaluated in triplicate.

Expression and Analysis of Data

The results of direct-binding studies measuring specific IgE anti-protamine antibodies were expressed as a binding ratio (the ratio of the counts per minute in unknown serum samples to the counts per minute in negative serum samples). The results of inhibition studies measuring anti-protamine IgE antibodies were expressed as the percentage of inhibition derived according to the following equation: (counts per minute in unknown serum samples incubated with buffer minus the counts per minute in unknown serum samples incubated with soluble protamine) divided by (counts per minute in unknown serum samples incubated with buffer minus the counts per minute in negative serum samples). For the ELISA and heterologous-interpolation assays, the standard dilution curve was fitted mathematically with the use of a three-parameter spline function as previously described.¹⁶ The antibody content of unknown serum samples was calculated from the curve function. For performance assessment, the ELISA precision profiles were calculated according to the method of Ekins.¹⁷ Positive serum samples were defined as those with a count per minute or optical density ≥ 2.5 times that of negative serum samples and inhibited ≥ 50 percent by soluble protamine. The odds ratios were calculated according to the method of Haldane,¹⁸ and their significance was assessed by Fisher's exact test. Confidence intervals were calculated with the use of exact conditional distribution.¹⁹ Because the odds ratio is the best estimate of the related relative risks, the latter term is used in this report. A comparison of the protamine IgG antibody levels between groups was done with use of the Kruskal-Wallis test followed by the Mann-Whitney U test. The Mann-Whitney U test was used to compare the duration of the reactions. All P values shown are two-tailed.

RESULTS

Radioallergosorbent Test for Protamine IgE

As shown in Figure 1, 9 of the 13 patients who reacted to protamine and who had been treated with subcutaneous protamine-insulin injections (Group E)

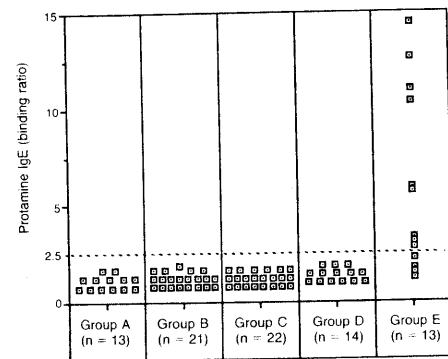


Figure 1. IgE Antibody to Protamine.

Serum levels of IgE antibody to protamine were measured with the use of the radioallergosorbent test in five patient populations. Normal subjects (Group A) had never been exposed to protamine in any form and included three atopic subjects with total serum IgE levels of more than 1000 ng per milliliter. Group B consisted of diabetic patients who had not received subcutaneous protamine-insulin injections, but who had received intravenous protamine after coronary-artery bypass surgery without a reaction. Group C consisted of diabetic patients who were receiving daily subcutaneous injections of protamine and insulin and who had no reaction to intravenous protamine after bypass surgery. Group D consisted of 13 nondiabetic patients and 1 diabetic patient who had adverse reactions to intravenous protamine but who had had no previous exposure to protamine-insulin preparations. Group E was composed of diabetic patients receiving daily subcutaneous injections of protamine and insulin who had adverse reactions when given intravenous protamine. The response was reported as a binding ratio (counts per minute of an unknown serum sample/counts per minute of a negative serum sample). A ratio of ≥ 2.5 indicated a positive response. Nine of the 13 patients in Group E had IgE antibodies to protamine, as compared with none of the 70 patients in the other four groups. Soluble protamine inhibited the binding of IgE antibody to the protamine-agarose complex in all nine positive serum samples. The inhibition of direct binding ranged from 60.7 to 99.5 percent (mean, 85.8 percent; data not shown).

had IgE antibodies to protamine. In contrast, none of the 57 patients in Groups B, C, and D had antiprotamine IgE antibodies. Soluble protamine competitively inhibited the binding of antiprotamine IgE antibodies to the protamine-agarose complex (range, 60.7 to 99.5 percent; mean, 85.8 percent) in all nine of the positive serum samples (data not shown). The specificity of the inhibition was confirmed by the inability of soluble protamine to inhibit the direct binding of antiragweed IgE antibodies to a ragweed-agarose sorbent (mean inhibition, 0 percent; $n = 4$ samples; data not shown). None of the 10 normal or 3 atopic subjects with elevated levels of total serum IgE (Group A) had increased binding in the protamine-agarose assay.

Reference Calibration and ELISA for Protamine IgG

Heterologous interpolation from a calibrated standard curve of antiragweed IgG antibody indicated that the serum from a diabetic patient who reacted to pro-

tamine had a mean ($\pm$ SEM) of $21.7 \pm 1.6 \mu\text{g}$ of antiprotamine IgG antibody per milliliter ($n = 2$ experiments). The protamine and ragweed curves were parallel, as reflected by an interdilutional coefficient of variation of only 12 percent. This calibrated reference serum was then used to generate an ELISA standard curve for the quantitation of antiprotamine IgG. Eleven of the 13 patients in Group E, 5 of the 14 patients in Group D, and 2 of the 22 patients in Group C had specific IgG antiprotamine antibodies (Fig. 2). The quantity of antiprotamine IgG antibodies was significantly higher in Group E (median, $36.4 \mu\text{g}$ per milliliter; $P < 0.0002$) or Group C (median, $0.1 \mu\text{g}$ per milliliter; $P < 0.0001$).

Association of Protamine Antibodies and Adverse Protamine Reactions

As shown in Table 1, in patients who had received subcutaneous protamine-insulin injections, serum antiprotamine IgE antibody was strongly associated with acute protamine reactions (relative risk, 95; $P = 1 \times 10^{-5}$), as was antiprotamine IgG antibody (relative risk, 38; $P = 1.2 \times 10^{-5}$). In patients who had never received protamine-insulin injections, antiprotamine IgG antibody was also associated with acute protamine reactions (relative risk, 25; $P = 0.0062$). No subject (case or control) without previous exposure to subcutaneous protamine-insulin preparations had IgE antibody to protamine.

Histories of Patients with Adverse Reactions to Protamine

The demographic features, types of reactions, and status with respect to specific antiprotamine antibodies of the 27 patients who had had reactions to intravenous protamine are shown in Table 2. Even with the exclusion of Patient 7, whose reaction lasted two days, the 13 patients (Patients 1 through 13) who had received subcutaneous protamine-insulin injections had reactions to intravenous administration of protamine that lasted longer than those of the 14 patients (14 through 27) who had never received subcutaneous injections ($P = 0.0024$). The protamine-insulin-dependent group also had reactions that tended to require more treatment and were more severe than those in the group that had not received such injections (Table 2). All patients with antiprotamine IgE antibody had reactions that were moderate to severe and involved hypotension, bronchospasm, or both (Table 2). Patients with only antiprotamine IgG antibody and those without demonstrable antiprotamine IgE or IgG antibodies had reactions involving elevations of pulmonary-artery pressure that were usually mild, but that could be severe (Table 2).

DISCUSSION

This study presents evidence that in patients with protamine-insulin-dependent diabetes, the increased risk of severe reactions after the intravenous administration of protamine is probably caused by antibody-mediated immune responses. The exact mechanisms

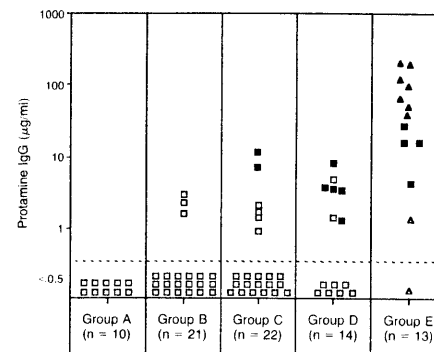


Figure 2. IgG Antibody to Protamine.

Levels of IgG antibody to protamine were measured with the use of ELISA in the five groups of patients (A through E). The open symbols indicate direct binding that was not inhibited (≥ 50 percent) by soluble protamine (5 mg per milliliter). The triangles indicate that serum also had IgE antibodies to protamine.

of adverse protamine reactions are incompletely understood. Although studies in animals initially suggested that protamine could cause the direct, nonimmunologic release of histamine from rat and hamster peritoneal mast cells *in vitro*,¹⁹ subsequent studies using human basophils and mast cells have not demonstrated histamine release with protamine at concentrations up to $100 \mu\text{g}$ per milliliter.²⁰ We have now shown that IgE antibodies to protamine may develop in patients with protamine-insulin-dependent diabetes that can result in the release of mediators from mast cells, basophils, or both through an immediate hypersensitivity reaction.²¹

It appears that protamine can induce more than one type of adverse response in humans. Some reactions may be associated with complement activation, either through protamine-heparin complexes or through an interaction between protamine and complement-fixing, antiprotamine IgG antibody. Protamine-heparin complexes have been shown *in vitro*²² and *in vivo*²³⁻²⁵ to activate complement through the classical pathway, with subsequent generation of anaphylatoxins C3a, C4a, and C5a. Recent evidence suggests that protamine could inhibit the action of plasma carboxypeptidase N, which cleaves the C-terminal arginine residue from the anaphylatoxins and bradykinin, converting them to their less active des-arginine metabolites.²⁶

These reactions may lead to the elevation of pulmonary-artery pressure and have been associated with the generation of thromboxane, a potent pulmonary vasoconstrictor.^{6,27-29} The elevation of pulmonary-artery pressure may be an isolated finding (Table 2), without clinical consequences. When severe, however, this pulmonary vasoconstriction may lead to acute right-sided heart failure, decreased filling of the left ventricle, systemic hypotension, and cardiovascular

collapse. In sheep and pigs, the rapid intravenous administration of protamine after heparin anticoagulation invariably produces increases in pulmonary-artery pressure and plasma levels of thromboxane B_2 ³⁰⁻³² that are associated with complement activation.²⁷ Pretreatment of the animals with inhibitors of cyclooxygenase or of thromboxane synthetase blocks the increase in plasma levels of thromboxane B_2 and pulmonary-artery pressure.³⁰⁻³² Since the anaphylatoxins can induce histamine release from human mast cells and basophils, cause neutrophil migration and release of polymorphonuclear-cell enzymes, initiate platelet aggregation, cause smooth-muscle contraction, and stimulate prostaglandin and thromboxane production by macrophages,^{33,34} it has been postulated that protamine reactions are caused by the generation of anaphylatoxins from the interaction of protamine and heparin.

Two lines of evidence suggest that complement activation from protamine-heparin complexes is probably not the mechanism by which most adverse protamine reactions occur in patients with protamine-insulin-dependent diabetes. First, complement activation induced by protamine and heparin has been shown to occur frequently in humans *in vivo* without adverse sequelae.^{24,25} Second, several studies have shown a 40-fold to 50-fold increase in the risk of protamine reactions in diabetic patients receiving daily subcutaneous injections of protamine and insulin.^{7,8} These studies, along with numerous reports^{2-5,10,27,28} of protamine reactions in such patients, suggest that previous immunologic sensitization may be largely responsible for protamine reactions in this high-risk patient population. The present study greatly strengthens the immunologic hypothesis. We found that in diabetic patients receiving protamine-insulin injections, the presence of protamine IgE and IgG antibodies was an important risk factor for protamine reactions, with relative risks of 95 and 38, respectively. Among patients who did react to intravenous protamine but who had no previous exposure to protamine-insulin injections, the presence of protamine IgG

Table 1. Association of Protamine Reactions and the Presence of Serum IgE and IgG Antibodies to Protamine.*

PROTAMINE-SPECIFIC ANTIBODY	CASES	CONTROLS	RELATIVE RISK	LOWER 95% CL	P VALUE
Exposure to subcutaneous protamine-insulin					
IgE					
Present	9	0			
Absent	4	22	95	6.6	1.0×10^{-5}
IgG					
Present	11	2			
Absent	2	20	38	5	1.2×10^{-5}
No exposure to subcutaneous protamine-insulin					
IgE					
Present	0	0			
Absent	14	21	NA	NA	0.99
IgG					
Present	5	0			
Absent	9	21	25	1.6	0.0062

*Cases were patients who had reactions; controls, patients who did not have reactions. CL denotes confidence limit, and NA not applicable.

Table 2. Demographic Features, Types of Reactions, and Protamine IgE and IgG Antibody Status in 27 Patients Who Had Reactions to Intravenous Protamine.*

PATIENT No.	AGE	SEX	PROCEDURE	REACTION				SUBCUTANEOUS PROTAMINE-INSULIN INJECTIONS	ANTIBODY TO PROTAMINE	
				TYPE	DURATION	TREATMENT	SEVERITY		IgE	IgG
yr	min	status (ratio)	status (μg/ml)							
1	63	F	CABS	↓ BP†	40	volume, epi, dop, nrg, steroids, H ₁	Severe	Yes	Pos (3.3)	Pos (200)
2	66	F	CABS	↓ BP, bronchospasm, rash	45	volume, epi, dop	Severe	Yes	Pos (3.1)	Pos (56.2)
3	53	M	CABS	↓ BP, bronchospasm	30	epi, H ₁ , steroids, phenylephrine, CaCl ₂ , dop, CPB	Severe	Yes	Pos (14.4)	Pos (156.6)
4	52	M	CABS	↓ BP	15	epi, H ₁ , volume, CaCl ₂	Moderate to severe	Yes	Pos (10.3)	Neg (<0.5)
5	40	F	CABS	Bronchospasm, urticaria	30	volume, epi, steroids, H ₁	Severe	Yes	Pos (11)	Pos (36.4)
6	71	M	CABS, AVR	↑ PAP, ↓ BP	<5	CaCl ₂ , epi	Mild to moderate	Yes	Neg (1.2)	Pos (3.8)
7	69	M	Cath	↓ BP, bronchospasm, rash	2 days	epi, volume, steroids, H ₁ , H ₂	Severe	Yes	Pos (12.6)	Pos (90)
8	32	M	AVS	↓ BP, CA	10	epi, ne, volume	Severe	Yes	Pos (2.6)	Pos (111)
9	70	M	PVS	↑ PAP, ↓ BP, CA	?	volume, epi, dop, CaCl ₂ , CPR	Severe	Yes	Neg (1.7)	Pos (24.5)
10	73	M	AVR	↑ PAP, ↓ BP	?	ne, lidocaine, CPB	Moderate to severe	Yes	Neg (1.5)	Pos (16)
11	57	F	MVR	↑ PAP, ↓ BP	?	Steroids, H ₁ , H ₂ , CPB	Severe	Yes	Neg (2.2)	Pos (15.9)
12	60	M	CABS	↓ BP, bronchospasm	10	ne, dop	Moderate to severe	Yes	Pos (5.9)	Neg (1.3)‡
13	67	M	CABS	↓ BP	>30	epi, ne	Severe	Yes	Pos (5.7)	Pos (48.1)
14	60	M	CABS	↑ PAP	2-3	None	Mild	No	Neg (0.9)	Neg (<0.5)
15	56	M	CABS	↑ PAP, ↓ BP	<5	CaCl ₂	Mild	No	Neg (1.6)	Pos (1.2)
16	50	M	CABS	↑ PAP	1-2	CaCl ₂	Mild	No	Neg (1.0)	Neg (<0.5)
17	64	M	CE	↓ BP	10	CaCl ₂ , volume, epi, phenylephrine	Moderate to severe	No	Neg (0.9)	Pos (3.3)
18	56	F	CABS	↑ PAP, ↓ BP	1	None	Mild	No	Neg (1.8)	Pos (3.2)
19	68	F	CABS	↑ PAP, ↓ BP, bronchospasm	10	ne, CPB	Severe	No	Neg (1.7)	Pos (8.0)
20	71	M	CABS	↑ PAP	2	epi	Mild	No	Neg (1.8)	Neg (<0.5)
21	22	M	AVR	↑ PAP, ↓ BP	1	Steroids	Mild	No	Neg (1.5)	Pos (3.5)
22	84	F	AVR	↑ PAP, ↓ BP, bronchospasm	<5	ne, dop, β-agonist	Moderate to severe	No	Neg (1.1)	Neg (<0.5)
23	64	M	CABS	↑ PAP, ↓ BP	5	ne, CaCl ₂	Mild to moderate	No	Neg (1.3)	Neg (<0.5)
24	54	M	Cath	Bronchospasm, urticaria	?	Steroids, H ₁	Mild	No	Neg (1.4)	Neg (1.3)‡
25	42	M	CABS	↑ PAP, ↓ BP, CA	15	CPR, steroids, epi, H ₁ , CaCl ₂ , CPB	Severe	No	Neg (1.5)	Neg (<0.5)
26	60	M	CABS	↑ PAP, ↓ BP	>30	epi, CaCl ₂ , phenylephrine, CPB	Severe	No	Neg (1.1)	Neg (<0.5)
27	62	M	MVR Cath	↑ PAP, ↓ BP CA	<5 <5	epi, CaCl ₂ epi, CaCl ₂ , CPR	Moderate Severe	No	Neg (1.0)	Neg (4.6)‡

*CABS denotes coronary-artery bypass surgery. ↓ BP a decrease in blood pressure (hypotension), epi epinephrine, dop dopamine, nrg nitroglycerin, H₁ histamine-1 receptor antagonist, CPB cardiopulmonary bypass, AVR aortic valve replacement, ↑ PAP an elevation in pulmonary-artery pressure, Cath cardiac catheterization, H₂ histamine-2 receptor antagonist, AVS aortovascular shunt, CA cardiac arrest, ne norepinephrine, PVS peripheral vascular surgery, CPR cardiopulmonary resuscitation, MVR mitral-valve regurgitation, and CE carotid endarterectomy.

†Patient 1 received intravenous protamine on two occasions, and hypotension occurred each time.

‡The antibody was not inhibited (≥50%) by soluble protamine.

antibody was a significant risk factor for protamine reactions (relative risk, 25; $P = 0.0062$); none of these patients had protamine IgE antibodies.

In any case-control study, potential sources of bias must be considered. In our study, the age and sex distributions of cases and controls were not significantly different (data not shown). Studies have suggested that injection through a central venous catheter was more likely to result in a reaction than was injection

through the left atrium or a peripheral vein,^{35,36} although protamine reactions clearly do occur with left-sided injections.³⁷ This potential source of bias was eliminated since all controls in our study received protamine through a central venous catheter. Because the controls were all patients undergoing coronary-artery bypass operations, for which large amounts of protamine are generally required, the lack of reactions in controls cannot be attributed to the administration

of small doses of protamine. The presence of antiprotamine antibody in 12 serum samples drawn at the time of the protamine reaction is evidence that in most if not all cases, protamine antibodies were present before the reaction.

IgE antibodies to protamine developed only in diabetic patients receiving daily subcutaneous injections of protamine-insulin, whereas IgG antibodies were also found in 5 of the 14 subjects who had reactions but did not have protamine-insulin-dependent diabetes. Although the IgG antibodies in the subjects who reacted to intravenous protamine but who had not been treated with protamine-insulin injections may have been naturally occurring cross-reacting antibodies, we consider it more likely that previous exposure to protamine during cardiac catheterization or vascular surgery induced an IgG antibody to protamine. It appears likely that isolated or infrequent exposure to protamine, as during cardiac catheterization or vascular surgery, is capable of stimulating an IgG immune response, but that frequent subcutaneous injections may be required to stimulate IgE antiprotamine antibodies. Although systemic reactions to daily subcutaneous injections of low doses of protamine have been reported,^{3,38} none of the diabetic patients who had reactions to intravenous protamine in our series reported reactions to their daily subcutaneous protamine-insulin injections, which contain only 1/1000 of the usual intravenous protamine dose. This supports the concept — described in patients allergic to penicillin — that the development of an adverse drug reaction depends in part on the dose and the route of administration.³⁹

We conclude that humorally mediated immune responses to protamine are the likely cause of the increased risk of reactions to intravenous protamine in patients with protamine-insulin-dependent diabetes. For patients who are not receiving such preparations, IgG antibody directed against protamine is associated with an increased risk of protamine reactions. It remains to be determined whether these antiprotamine IgG antibodies are causally related to protamine reactions. A prospective trial evaluating the predictive value of specific antiprotamine antibodies for subsequent protamine reactions should be undertaken; yet such a prospective study is not likely in view of the serious risk of anaphylaxis suggested by the presence of IgE antibody. Even if the positive predictive value for anaphylaxis is only 50 to 70 percent, as with penicillin⁴⁰ and Hymenoptera⁴¹ allergies, the prescreening of high-risk patients, such as diabetic patients receiving protamine-insulin injections, before elective procedures that would normally involve the administration of intravenous protamine may be worthwhile. If such antibodies are present, special precautions could be taken, or other heparin antagonists, such as hexadimethrine, could be substituted for protamine.³

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PERSISTENCE OF IMMUNITY TO VARICELLA IN CHILDREN WITH LEUKEMIA IMMUNIZED WITH LIVE ATTENUATED VARICELLA VACCINE

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Abstract To determine the safety and efficacy of varicella vaccine, we studied 437 children in remission from leukemia who were immunized with live attenuated varicella virus. Three hundred one of the patients received two doses of vaccine and 136 received a single dose of vaccine from 1 of 10 lots from two manufacturers. The patients have been followed for an average of three years (range, one to six).

Seroconversion occurred in 88 percent of the 437 children after the first dose of vaccine and in 98 percent after one or two doses. The proportions of patients who were seronegative after one, three, and five years were 20, 25, and 30 percent, respectively, with little change over time in the geometric mean titers of specific antibody (6.3, 6.5, and 5.7, respectively). Chickenpox has been documented in 36 vaccinated patients (8 percent) who had 3 to 640 vesicles (mean, 100), mild illness, and no complications. Of the 83 vaccinated patients exposed to varicella within

their families, 11 had chickenpox; the attack rate was 14 percent (8 percent among seropositive patients, 29 percent among seronegative patients). There was no relation between the time since vaccination and either the attack rate or the severity of the breakthrough illness. Two doses of vaccine appeared to be no more effective than a single dose.

Of the 372 patients receiving maintenance chemotherapy when immunized, 149 (40 percent) had a rash, which was treated with acyclovir in 16 children (4 percent) and became a severe febrile illness in 4. These reactions were not fatal and were all associated with vaccine lots, the use of which has since been discontinued.

We conclude that in children in remission from leukemia, varicella vaccine is safe and induces an immunity to chickenpox that persists for more than three years. (*N Engl J Med* 1989; 320:892-7.)

chemotherapy.¹ Varicella in otherwise healthy children may also have serious health and economic consequences.² A live attenuated varicella vaccine was developed in the early 1970s in Japan.³ Although this

vaccine has been administered to over 15,000 people, including healthy children,⁴ healthy adults,⁵ and certain immunocompromised patients,⁶ it is not licensed in the United States. It has been licensed, primarily for immunocompromised varicella-susceptible children, in Japan and in some countries in Europe. Two major uncertainties relevant to the licensing of varicella vaccine in the United States are the incidence of reactivation of varicella-zoster virus after vaccination and the duration of immunity to chickenpox. Studies have indicated that after immunization, the incidence of zoster among children with leukemia is not higher than that in matched controls and may be lower.⁷⁻⁹ This report addresses the second concern, that of the duration of immunity.

METHODS

Four hundred thirty-seven children with leukemia in remission for one year or more were immunized with the Oka strain of live attenuated varicella vaccine. Sixty-five had stopped receiving treatment for leukemia within the previous 1 to 12 months at the time of immunization. In the 372 others, maintenance chemotherapy was withheld one week before and one week after vaccination. The children were immunized by the Varicella Vaccine Collaborative Study Group of the National Institute of Allergy and Infectious Diseases between 1979 and 1988. They received vaccine from one of three sources: one of three vaccine preparations derived from the Oka strain of live attenuated varicella vaccine, provided by Smith-Kline RIT (Rixensart, Belgium), which contained approximately 1000 to 2000 plaque-forming units per dose; one of two research lots prepared by Merck (West Point, Pa.), containing either 1400 or 2350 plaque-forming units; or one of seven production lots made by Merck that contained 1000 to 4000 plaque-forming units. In 1985, 96 children received one of five early production lots among the seven made by Merck. Although there were no differences in the clinical reactions in healthy children and adolescents who were immunized with vaccine from these five lots, the children with leukemia who received that vaccine had a higher rate of rash than had previously been observed¹⁰ (and Lydick et al.; personal communication). These vaccine lots were found to contain a higher ratio of viable virus to viral protein than Merck's research lots.¹¹ Of the remaining 341 vaccinated patients, 290 received one of Merck's research lots or later production lots between 1981 and 1988, and 51 received vaccine from Smith-Kline between 1980 and 1981; both vaccines resulted in similar immunologic and virologic responses.⁶

At this writing, approximately two thirds of the vaccinated patients are still being followed. Originally a one-dose schedule of immunization was intended, but when only about 85 percent of the patients had antibodies after one dose and some low detectable antibodies within months, a two-dose regimen (with the doses usually separated by three months) was instituted. Maintenance chemotherapy was suspended for the first dose of vaccine. Because of the change in the protocol, only 301 of the children received two doses; 136 received a single dose of vaccine. The two-dose schedule was recommended in 1982; by 1983 there was excellent compliance. A second dose was given to both seronegative and seropositive patients. Up to four doses of vaccine were also administered to 20 patients who continued to receive chemotherapy for leukemia for more than one year after the last dose of vaccine had been given, and to seven who either did not undergo seroconversion or had minimal antibody responses (e.g., a titer of 1:4 for only one to two months) even after two doses of vaccine. There have been 42 deaths among the vaccinated patients, all due to complications of acute leukemia; none was related to varicella-zoster virus. Eighty-two patients (19 percent) had a relapse of leukemia after immunization. In a matched control group of 90 children with leukemia who had natural varicella,¹² 19 (21 percent) had a relapse of leukemia. In the subset of matched patients with leukemia who were vaccinated, 22 of 90 (24 percent) had a relapse.

The vaccinated patients were followed for possible adverse effects of the vaccine and for assessment of their immunity to varicella-

zoster virus. The antibody titers were measured monthly for eight months and every four months thereafter with use of the fluorescent antibody-to-membrane-antigen assay.¹³ The measurement of cell-mediated immunity against varicella-zoster virus was also performed initially but was discontinued, because it was expensive and did not yield additional important immunologic information. The efficacy of vaccination was considered to have been demonstrated if the patients who received it were protected from chickenpox after proved exposures to varicella in their families. If a sibling was given a diagnosis of varicella, the disease was considered to be chickenpox if varicella-zoster virus was isolated from the sibling or from the vaccinated patient, if a breakthrough illness developed, or if the serologic response consisted of an increase in the antibody titer to the virus of fourfold or more in the family member or in the vaccinated patient after the exposure. All the virus isolates were typed by restriction-endonuclease analysis to determine if they were wild type or vaccine type.¹³ Vaccinated patients who had varicella despite immunization were examined clinically to determine the number of skin vesicles they had and to identify any signs of systemic illness, such as fever. Virus isolates were obtained from vesicular rashes, and antibody titers were measured before and after exposures to varicella or zoster.

RESULTS

Seroconversions and Rash after Vaccination

After the first dose of vaccine, 384 of the 437 children (88 percent) underwent seroconversion with respect to varicella-zoster virus. Of the 65 children who were no longer receiving chemotherapy, 62 (95 percent) underwent seroconversion. Of the 372 children whose maintenance chemotherapy was suspended for the vaccination, 322 (87 percent) underwent seroconversion. The differences in these rates of seroconversion are not statistically significant ($P>0.05$ by chi-square analysis with Yates' correction). In addition to the 248 children who had undergone seroconversion but nevertheless received a second dose of vaccine, 52 of the 53 children who did not undergo seroconversion after one dose received a second dose, and 45 of them (86 percent) then underwent seroconversion. Hence, the overall seroconversion rate was 98 percent. Of the 301 children who received two doses, 7 (2 percent) did not undergo seroconversion. Seven of the 65 children no longer receiving chemotherapy (11 percent) had a rash that was temporally related to immunization. One hundred forty-nine of the 372 children whose chemotherapy was suspended for the vaccination (40 percent) had the same type of rash. This difference was significant ($P<0.001$ by chi-square analysis with Yates' correction). The rashes were observed almost exclusively after the first dose of vaccine. A vaccine-type virus was isolated from 17 children between 7 and 40 days (mean, 28) after the first dose. The transmission of a vaccine-type virus to a sibling, observed in 12 instances, occurred exclusively in vaccinated patients who had a rash.⁶ A vaccine-associated rash was severe in four children, who had 500 to 1000 skin lesions. These children also had fever with temperatures to 41°C, hemorrhagic skin lesions, and abdominal and back pain; one had radiologic evidence suggestive of pneumonia. They were treated with acyclovir, and all recovered. These children were among the 96 who had received vaccine in 1985 from Merck lots that are no longer in use. The rate of rash with this vaccine among children receiving maintenance chemotherapy

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