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Complement Activation From Protamine Sulfate Administration After Coronary Angiography

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The cause of hypotension after reversal of heparin by protamine has not been well defined. In this study we evaluated complement activation (C3a and C4a) by the heparin-protamine complex in 46 consecutive patients (40 received protamine sulfate to reverse heparin, and six did not) during and after coronary angiography. In patients receiving protamine sulfate, there was a significant increase in C3a over the value before protamine sulfate administration ($P < .001$) or in patients who did not receive protamine sulfate ($P < .05$): 807 ± 100 ng/ml vs. 274 ± 75 ng/ml. There were no significant changes in C4a after protamine sulfate administration. These results indicate that the alternate complement pathway is activated when protamine sulfate is administered after coronary angiography. This may induce hypotension as well as platelet aggregation and thrombus formation and may contribute to coronary instability. Therefore, in unstable patients, heparin reversal by protamine should not be done routinely.

Key words: heart catheterization; heparin-protamine complex, complement; angina, unstable

INTRODUCTION

Protamine, a polycationic peptide obtained from salmon sperm or testes, is used to reverse the anticoagulation produced by the heparin administered during various cardiac and vascular angiographic procedures. The number of reported incidences of adverse reactions to protamine sulfate administration has increased over the past several years, probably because of the increasing number of invasive procedures being performed [1]. These adverse reactions have been summarized by Weiler et al. [2] and include skin rash, urticaria, hypotension, respiratory difficulties, leukopenia, thrombocytopenia, shock, and death. The most common cardiovascular reaction is mild transient hypotension [1]. Although the severity may be lessened by slow administration of protamine sulfate, hypotension still may develop in some patients.

Patients who undergo coronary angiography while they are unstable (severe coronary artery or valvular disease) may be at an increased risk of more severe complications secondary to hemodynamic fluctuations after protamine administration. These hemodynamic changes may be induced by immunologically mediated mechanisms [3–8]. This may be particularly true for patients who have had prior exposure to protamine in previous vascular procedures or for diabetic patients taking NPH insulin [9]. However, more recent evidence suggests that the use of NPH insulin is not associated with an increased incidence of anaphylactoid reactions to protamine sulfate [10]. In addition, those sensitized because of crossreacting antibodies from a fish allergy [4,11] or prior vasectomy [12,13] may be at increased risk. Complement activation has also been implicated. These reactions may occur as a result of direct activation of complement by the heparin-protamine complex [14,15] or through complexes formed with C-reactive protein, which in turn activate complement [16]. This latter pathway may play a role in patients who have not had prior exposure to protamine. In addition, the role of complement activation during cardiopulmonary bypass and subsequent heparin reversal has been described [17–19].

Best et al. [20] described a case of anaphylactoid reaction to protamine sulfate and demonstrated that complement was activated via the classic pathway. However, studies that specifically evaluated complement activation in a group of patients undergoing coronary angiography have not been reported. Therefore, we evaluated complement activation in patients undergoing coronary angiography and subsequent reversal of heparin with protamine.

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TABLE I. Clinical Characteristics and Angiogram Results*

	Group I (protamine)	Group II (no protamine)
No.	40	6
Age (y)	63 ± 1.8	63 ± 3
Sex distribution	28 M, 12 F	6 M
Reason for angiogram		
Chest pain	22	2
Unstable angina	7	1
Valve disease	4	1
Recent MI	4	1
Cardiomyopathy	2	0
VT/VF	1	1
Angiogram results		
LVEF (%)	53 ± 2	56 ± 2
Valvular disease		
Mitral regurgitation	11 (mild)	1 (severe) 1 (moderate)
Mitral stenosis	1 (moderate)	0
Aortic stenosis	1 (moderate) 1 (severe)	0
Aortic insufficiency	2 (mild)	0
Wall motion abnormalities		
Mild	20	3
Moderate	3	2
Severe	6	0
Coronary artery disease		
None	6	1
Mild	7	1
Moderate	15	1
Severe	12	3

*Abbreviations: F, female; LVEF, left ventricular ejection fraction; M, male; MI, myocardial infarction; VT/VF; ventricular tachycardia or fibrillation.

MATERIALS AND METHODS

Patient Selection

Forty-six patients referred for routine coronary angiography were studied. Because we were measuring complement activation, which would be more reflective of nonimmunologic heparin-protamine interaction, patients with prior exposure to protamine, diabetics taking NPH insulin, and patients with a history of fish allergy or prior vasectomy were not included. Informed consent for blood sampling during the procedure was obtained for each patient.

Study Protocol

All patients underwent routine cardiac catheterization (percutaneous femoral technique) including left ventriculography and coronary arteriography. All received heparin sulfate, 2,500 units intravenously, after insertion of the femoral artery catheter. Blood samples for analysis of complement activation (C3a and C4a), activated partial thromboplastin time, platelet and leukocyte counts, and hemoglobin were drawn from the arterial catheter during the procedure at the following times: 1) prior to administration of heparin sulfate, 2) after administration of heparin but prior to the left ventriculographic procedure, 3) after the left ventriculogram and coronary angiogram were obtained, and 4) 10 min after reversal of heparin with protamine sulfate (25 mg, intravenously). There were two groups of patients. Group I, 40 patients who received protamine sulfate as described, were the study group with intragroup comparison at different times as the control. In group II were six patients who were treated similarly but were not given protamine sulfate, for an additional comparison. In group II, a femoral sheath was left in place, and protamine sulfate was not given because of severe coronary artery disease and the potential for instability if the patient became hypotensive. Arterial blood pressure was monitored continuously in all patients via the arterial catheter during the procedure. After removal of the femoral artery catheter, cuff blood pressure was taken at 10, 15, 30, and 45 min.

Complement Analysis

After they were drawn, the blood samples were immediately placed in disodium ethylenediaminetetraacetate tubes, centrifuged in a refrigerated centrifuge for 10 min, and frozen at −70°C. Complement activation was measured by radioimmunoassay of plasma C3a and C4a with the Upjohn Diagnostics analysis kit [21,22].

C3a was used as a marker of both the classic and the alternate complement cascade activation, and C4a indicated activation of complement via the classic pathway.

Blood samples for the other analyses were immediately sent to the hospital hematology laboratory and analyzed in the routine manner.

Statistical Analysis

Continuous variables for independent groups were compared by *t*-tests for two groups or two-way analysis of variance and within groups by the Newman-Keuls method [23]. Simple linear regression analysis was used to compare blood pressure changes with changes in complement. Data are expressed as mean ± SEM. $P < .05$ was considered to indicate statistical significance.

RESULTS

Table I summarizes the clinical characteristics and angiographic results for both groups. None of the patients in group I had a severe clinical response to protamine sulfate administration. Nineteen patients in group I had a decrease in blood pressure of at least 10 mm Hg after protamine sulfate administration. In addition, one patient in group I had a decrease of 40 mm Hg (110 mm Hg systolic to 70 mm Hg systolic). This patient's laboratory data are presented in Table II and were not included in the overall analysis because of the marked increase in

TABLE II. Laboratory Values and Blood Pressure for One Group I Patient With Significant Hypotension After Protamine Sulfate Administration*

Sample	APTT (s)	Platelets (No. × $10^3/\mu l$)	WBC (No. × $10^3/\mu l$)	Hb (g/dl)	C3a (ng/ml)	C4a (ng/ml)	BP (mm Hg)
Before protamine							
1	39	271	11.7	11.1	357	603	110
2	>100	268	11.8	11.1	308	547	115
3	>100	214	10.6	10.4	245	427	112
After protamine							
4	39	218	8.4	10.3	10,110	437	100[a]

*Abbreviations: APTT, activated partial thromboplastin time; BP, blood pressure; Hb, hemoglobin; WBC, leukocytes.

[a]Subsequent readings were 70 mm Hg at 15 min, 76 mm Hg at 30 min, and 78 mm Hg at 45 min.

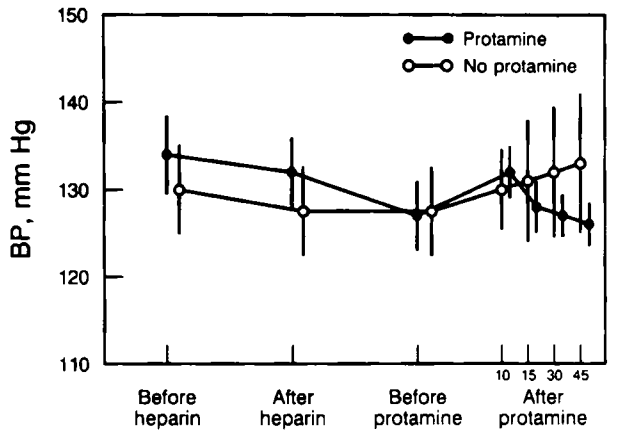

Fig. 1. Mean (± SE) systolic blood pressures (BP) of both patient groups during the study.

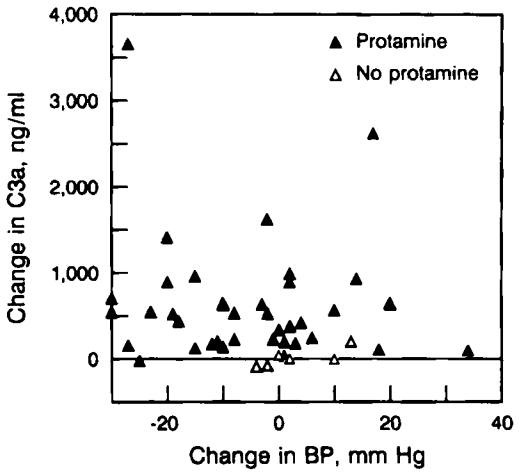

Fig. 2. Correlation between change in C3a and blood pressure (BP) for group I. The change was determined from values just prior to protamine sulfate administration and 15 min after administration.

C3a after protamine sulfate administration. One patient in group II had a decrease in blood pressure of 15 mm Hg in the recovery period. Although there was a trend toward a decrease in systolic blood pressure after protamine sulfate administration in group I, the difference between the groups was not statistically significant (Fig. 1).

The laboratory results for all patients are presented in Table III. After protamine sulfate administration there was a significant increase in C3a in group I compared to values prior to protamine administration in that group ($P < .001$) and all values in group II ($P < .05$). There was no significant difference in the C4a levels between groups. In fact, there was a trend for a decrease in C4a level during the study; the decrease in group I between the initial value and the value after protamine sulfate administration was significant. There was no significant difference in complement levels between men and women in group I. In the one patient in group I who had marked hypotension, after protamine sulfate administration the C3a level increased almost 30-fold (Table II).

For group I, linear regression analysis comparing changes in C3a level with changes in blood pressure did not show a significant relationship (Fig. 2). However, nearly one-half of the patients in group I had a decrease in blood pressure of at least 10 mm Hg after protamine sulfate administration. In addition, in group I there was a significant decrease in leukocyte count after protamine sulfate administration. There was a significant decrease in platelet count at the end of the study, but this could not be directly attributed to protamine, although we did not see a similar response in patients not receiving protamine sulfate. The increase in C3a level without an increase in C4a indicates complement activation via the alternate pathway.

DISCUSSION

Best et al. [20] reported a case in which a patient had a severe anaphylactoid reaction after cardiopulmonary

TABLE III. Laboratory Values for All Patients, by Group*

Sample[a]	APTT (s)	Platelets (No. × $10^3/\mu l$)	WBC (No. × $10^3/\mu l$)	Hb (g/dl)	C3a (ng/ml)	C4a (ng/ml)
Group I (n = 39)						
1	29 ± 0.6	266 ± 11	6.9 ± 0.3	13.3 ± 0.2	291 ± 30	995 ± 188[b]
2	95 ± 1.8	265 ± 12	6.9 ± 0.3	13.4 ± 0.3	219 ± 18	519 ± 72
3	96 ± 1.6	249 ± 10	7.1 ± 0.3	12.7 ± 0.3	168 ± 11	461 ± 49
4	33 ± 1.2	229 ± 11[c]	5.9 ± 0.3[d]	12.6 ± 0.3	807 ± 110[e]	587 ± 64
Group II (n = 6)						
1	33 ± 1.6	262 ± 26	8.8 ± 2	12.1 ± 0.5	421 ± 130	834 ± 103
2	97 ± 3.2	292 ± 40	8.8 ± 2	12.3 ± 0.5	372 ± 102	738 ± 112
3	95 ± 3.4	280 ± 35	8.8 ± 2	11.7 ± 0.5	253 ± 90	600 ± 93
4	83 ± 6.1	272 ± 35	8.3 ± 2	11.6 ± 0.5	274 ± 75	517 ± 87

*The data are shown as mean ± SE. Abbreviations are as in Table II.
[a] 1, Before heparin; 2, after heparin; 3, before protamine; 4, after protamine.
[b] $P < .05$ for intragroup comparison.
[c] $P < .05$ for intragroup, sample 4 vs. 1 & 2.
[d] $P < .05$ for intragroup, sample 4 vs. 3.
[e] $P < .001$ for intragroup comparison; $P < .05$ for intergroup comparison.

bypass and protamine sulfate administration. Measurement of complement and fibrin split products indicated activation of complement via the classic pathway. Cavarocchi et al. [19] demonstrated complement activation via the alternate pathway during cardiopulmonary bypass and a second activation of complement via the classic pathway after protamine sulfate administration. Despite the extensive literature regarding cardiopulmonary bypass, the immunologic interactions and role of complement activation remain controversial [17,18,24–26]. In addition, these previous studies included patients with diabetes mellitus, and presumably all had had prior exposure to protamine during coronary angiography prior to the bypass. The subsequent effect of protamine sulfate administration on complement activation is uncertain. In our study design, the complement activation from cardiopulmonary bypass or prior protamine exposure was not a factor.

The hemodynamic effects of protamine sulfate administration have been documented [27,28]. The hypotension is primarily the result of peripheral vasodilatation. The mediators of this hypotension may be derived from the release of anaphylatoxins induced by complement activation. These anaphylatoxins can cause a response similar to an inflammatory reaction, with vasodilatation and histamine release from mast cells and leukocytes [28–31]. Frater et al. [28] found an increase in histamine after reversal of heparin with protamine sulfate injected via the right atrium. Although we could not demonstrate a significant hypotensive effect in the entire group of patients receiving protamine sulfate, nearly one-half of them had a decrease in blood pressure greater than 10 mm Hg after the protamine sulfate was given. The patient with the marked increase in C3a had the largest decrease in blood pressure.

Thrombocytopenia [32–35] and transient neutropenia [36] after protamine sulfate administration have been described. We observed a significant decrease in leukocytes in patients receiving protamine sulfate. When complement is activated, leukocytes are activated, clump, and adhere to vessel walls [35]. In addition, this study suggests an effect on platelets as well. Presumably, the thrombocytopenia is secondary to platelet activation and clumping by complement and anaphylatoxins; however, the mechanisms whereby platelets participate in the adverse response to protamine are unknown.

The vast majority of patients who undergo coronary angiography in which anticoagulation with heparin sulfate and subsequent reversal with protamine sulfate is performed have no serious consequences. However, the potential for serious complications secondary to complement activation—hypotension, histamine-induced epicardial coronary artery constriction [36], platelet aggregation in critical stenotic segments, and leukocyte activation—may be greatest in patients who have severe coronary artery or valvular disease.

In our own practice, patients with critical coronary artery stenosis (e.g., >90% stenosis) have developed unstable angina after the protamine sulfate injection used to reverse heparin anticoagulation. It may be reasonable to avoid reversal of heparin in these patients, using instead an arterial sheath to prevent bleeding and allowing the anticoagulant effect to be reversed by the metabolism of the heparin. The rationale for this approach is supported by the results of this study demonstrating complement activation by protamine and the potential adverse effects.

We chose to evaluate patients who had had no prior exposure to protamine, to avoid possible immunologically mediated effects. Patients who have a history of

prior protamine exposure, of fish allergy, of NPH insulin use, or vasectomy may have an additional risk when given protamine sulfate.

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