

C5a and Thromboxane Generation Associated with Pulmonary Vaso- and Broncho-constriction during Protamine Reversal of Heparin

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The authors conducted a study in humans to determine the mediators associated with acute pulmonary vaso- and broncho-constriction occurring episodically with protamine reversal of heparin anticoagulation. Of 48 adult patients investigated prospectively after termination of cardiopulmonary bypass, two presented a sudden increase of airway pressure, acute pulmonary hypertension, and systemic hypotension 1-3 min after right atrial protamine injection. In these two subjects, plasma levels of C5a increased from 0.7 and 2.2 to 9.8 and 9.9 ng/ml, respectively, and thromboxane B₂ increased from 0.26 and 0.34 to 7.5 and 16.2 ng/ml 1 minute after drug injection. A third subject not identified prospectively had an identical reaction and mediator profile (C5a, 10.2 ng/ml; TxB₂, 18.6 ng/ml at 1 min). The plasma levels of these mediators were unchanged in the remaining patients (C5a, 0.7 ± 1.1 [x ± S.D.] to 0.6 ± 0.9 ng/ml; TxB₂, 0.16 ± 0.12 to 0.15 ± 0.07 ng/ml). Plasma histamine was not involved in this type of reaction, but increased from 0.7-10.4 ng/ml in a fourth patient who became hypotensive without acute pul-

monary hypertension, bronchoconstriction, or elevation of C5a or TxB₂. The authors' data indicate that the generation of high plasma levels of C5a anaphylatoxins and thromboxane is associated with pulmonary vaso- and broncho-constriction induced by protamine reversal of heparin in humans. (Key words: Bronchoconstriction. Complications, protamine: bronchoconstriction; hypotension; pulmonary hypertension; right ventricular failure. Heparin-protamine complexes. Mediators, anaphylatoxins, complement: C3a; C5a. Mediators, anaphylatoxins, histamine. Mediators, anaphylatoxins, prostaglandins: thromboxane; prostacyclin. Pulmonary leukocyte sequestration. Pulmonary vasoconstriction. Thrombocytopenia.)

THIS PROSPECTIVE STUDY was conducted to determine the mediators associated with catastrophic pulmonary vasoconstriction^{1,2} and bronchoconstriction^{2,3} occurring episodically with protamine reversal of heparin. We reasoned that the pulmonary vascular and airway constriction was most likely due to liberation of thromboxane A₂ (TxA₂). These substances are among the most potent known pulmonary vasoconstrictors in animal models.⁴⁻¹¹ Although thromboxane can be synthesized by a variety of human cells,¹²⁻¹⁵ it has not been established that it can affect pulmonary vascular and airway tone in man.

Injecting activated complement into animals causes release of thromboxane by cells within the lung, resulting in transient pulmonary vasoconstriction and hypoxemia.^{4,5} *In vitro* formation of heparin-protamine complexes activates the complement cascade nonimmunologically *via* the classical pathway.¹⁶ Two recent prospective studies in humans have demonstrated anaphylatoxins C3a and C4a, but not C5a, generation during administration of protamine to achieve heparin reversal immediately after cardiopulmonary bypass.^{17,18} No patients in these studies sustained an acute pulmonary hypertensive reaction, despite complement activation. Since the adverse reactions to protamine administration are believed to be of allergic origin,^{19,20} we further hypothesized that histamine liberation, an integral component of true anaphylaxis, was probably uninvolved in the pulmonary vasoconstriction response. We therefore measured hemodynamics, airway pressure, and plasma concentrations of complement anaphylatoxins, thromboxane B₂ (TxB₂) and related mediators, and histamine, before and immediately after protamine sulfate injection into heparinized patients following cardiopulmonary bypass for cardiac surgery, with the

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hope of determining whether a different and characteristic mediator profile was present in patients demonstrating this unusual adverse response.

Methods

PATIENT POPULATION AND SURGICAL PROCEDURES

Forty-eight adult patients scheduled to undergo cardiac surgery utilizing cardiopulmonary bypass were selected arbitrarily and studied prospectively after obtaining informed consent. The first 26 were studied with a comprehensive protocol, which required cessation of surgery for 15 min, and allowed definition of the mediator profile normally associated with heparin neutralization. The last 22 were studied with a modified protocol which required only a 6-min interruption of surgery unless a reaction occurred. Two of these 48 patients developed pulmonary hypertension, increased airway pressure, and systemic hypotension following protamine administration. Data from one other (49th) patient, who was not selected for study in advance, are also presented. Blood sampling from this patient was initiated as soon as increased airway pressure and pulmonary hypertension were recognized, 1 min after administration of protamine. Thus, a total of 49 patients are reported in this study.

The group studied comprehensively consisted of 26 patients undergoing elective coronary artery bypass grafting (CABG, $n = 17$) or having a mitral valve replacement (MVR, $n = 9$). They ranged from 32–75 yr of age (mean \pm S.D., 61 ± 10). Patients with a documented hypersensitivity to protamine were excluded. Most of the CABG patients were being treated with beta-adrenergic blocking agents and long-acting nitrate preparations; seven were, in addition, under calcium-entry blocker treatment. These medications were continued to the day of surgery. Four patients were diabetics who had been treated with neutral protamine Hagedorn (NPH) insulin for over a year. No patients had received a nonsteroidal or steroidal antiinflammatory agent during the 2 weeks prior to operation. One of these 26 patients evidenced transient acute pulmonary hypertension following protamine injection.

In the 22 patients studied with the modified protocol, we withdrew a control blood sample prior to protamine administration and recorded the clinical hemodynamic measurements (c.f. below). If no adverse cardiopulmonary response occurred, the sample was discarded. If an increase in airway or pulmonary arterial pressure was noted, we drew additional blood samples and measured each variable over the same time period as in the initial group. Only one episode of pulmonary hypertension occurred among these 22 patients.

Anesthetic regimen was uniform in all patients, and consisted of high doses of fentanyl and diazepam, plus a

mixture of nitrous oxide in oxygen. Pancuronium bromide was used for neuromuscular blockade. Cardiopulmonary bypass employed a Sarns roller pump, a Travenol TMO® membrane oxygenator, and Pall filters. The pump prime solution consisted of crystalloid solution with 5% albumin. Before vascular cannulation and institution of bypass, bovine-lung heparin (Organon) was administered intravenously in a dose ranging from 290–430 IU per kilogram (mean \pm S.D., 340 ± 50). Moderate hypothermia (27–29° C), partial hemodilution, and cold potassium cardioplegia were used in all patients. Additional doses of heparin were infused to maintain the activated clotting time (ACT) over 400 s.

The study, which lasted from 5 min before to 10 min after protamine administration, was conducted 10–20 min following separation from cardiopulmonary bypass, when hemodynamic stability was established. During the study, all supportive measures were maintained at constant levels, no blood was transfused, surgical manipulation of the heart was discontinued, and mechanical ventilation with oxygen was controlled by a volume ventilator at a tidal volume of 12 ml per kilogram. Protamine sulfate (Lilly) was injected over a 10-s period into the right atrium. All patients received 100 mg of protamine, except those with mitral valve disease and preexisting pulmonary hypertension, who were given 20 mg. This lower dose was motivated by our previous experience,¹ suggesting that patients with mitral valve disease (and the preexisting accompanying pulmonary vascular disease) might respond more severely with protamine-associated pulmonary vasoconstriction than patients with isolated coronary artery disease.

HEMODYNAMIC AND AIRWAY MEASUREMENTS AND BLOOD COLLECTION

Arterial blood pressure, pulmonary arterial pressure, and central venous and left atrial pressures were continuously monitored by calibrated pressure transducers (Gould T23ID), with a zero reference taken at the level of the right atrium determined in the open chest, and recorded on an eight-channel recorder (Hewlett-Packard 7758). Cardiac output was measured in duplicate by thermodilution. Systemic vascular resistance and pulmonary vascular resistance were calculated by standard formulae. Peak inspiratory airway pressure was read from a manometer and recorded at each blood sampling time.

Ten milliliters of arterial blood and 10 ml of mixed venous blood were drawn simultaneously from the radial artery and the pulmonary artery catheters at the following times: 5 min after the initial heparin administration (before bypass), 5 and 1 min prior to, and then 1, 3, 5, and 10 min following, protamine injection. Cardiac output and mean blood pressure was measured at the same time intervals. Blood samples for Tx_{B2}, prostaglandins, his-

tamine, and complement fragments were collected in plastic syringes and transferred to previously chilled glass test tubes placed on ice and centrifuged within 1 h at 2500 g at 4° C for 10 min. Plasma was aspirated and stored in polypropylene tubes at -80° C. Test tubes to assay TxB₂, prostaglandins, and complement contained EDTA as the anticoagulant. Tubes for histamine analysis contained heparin. In addition, immediately before blood sampling, 100 micrograms of indomethacin were added to the tubes scheduled for TxB₂ and prostaglandin determination. Total white blood cell and erythrocyte count, hematocrit, hemoglobin, and platelet concentration were measured by automated Coulter counter (Coulter Electronics, Hialeah, Florida). The ACT of whole blood was measured by a Hemocron 400D system (International Technidyne, Edison, New Jersey). The ACT values were obtained before and 5 min after the initial dose of heparin, and again before and 10 min after protamine injection. Individual heparin dose-ACT response slopes were calculated by the method of Bull *et al.*,²¹ and used to determine the amount of heparin which was neutralized by protamine administration.

BIOCHEMICAL ANALYSES

Complement. Plasma levels of the complement-derived anaphylatoxin antigens for C3a, C4a, and C5a were determined by radioimmunoassay procedures,²² employing kits obtained from Upjohn Diagnostics (Kalamazoo, Michigan).

Eicosanoids. Plasma levels of TxB₂, 6-keto-PGF_{1α}, and PGE₂ were determined by radioimmunoassays, as described previously,²³ using antisera provided by Dr. L. Levine.²³ The assays were standardized by TxB₂, 6-keto-PGF_{1α}, and PGE₂ obtained from Upjohn Diagnostics (Kalamazoo, Michigan). Further evidence for the identification of TxB₂ was obtained by reverse phase high performance liquid chromatography, demonstrating that plasma TxB₂ immunoreactivity had the chromatographic mobility of authentic TxB₂.

Histamine. Plasma histamine levels were determined with the use of the radioenzymatic assay described by Bowsher *et al.*²⁴ The inter- and intra-assay coefficient of variation was 8%.

All samples for biochemical analyses were stored at -80° C and assayed within 3 months of collection. Both arterial and mixed venous plasma samples were measured.

DATA ANALYSIS

A one-way analysis of variance was used to detect statistical differences of variables over time between pre- and post-protamine data, and the pre-bypass measurements were compared to post-bypass data by Student's paired *t* test corrected for multiple comparisons. A two-

tailed *P* value of <0.05 was considered significant. The complement and histamine data were statistically analyzed after being subjected to logarithmic transformation, which provided a better approximation of a normal distribution of the data at the different time intervals. Correlation between variables was determined by linear regression analysis, and Pearson's correlation coefficient was calculated.

Results

HEMODYNAMIC VARIABLES AND AIRWAY PRESSURE

Three patients demonstrated an acute increase in pulmonary arterial pressure (+54, +69, +155% increase over control values), immediately following 100 mg protamine administration (table 1). This was followed by systemic hypotension requiring treatment with CaCl₂ and phenylephrine. Figure 1 shows the time course of the reaction in one patient. A rise in peak airway pressure preceded the pulmonary vasoconstriction in all reactors and never occurred in any other patient (fig. 2).

Two of the three responding patients were diabetics who had received NPH insulin in the past. Hemodynamic measurements in these three reacting subjects before operation and prior to protamine injection were normal, including mean pulmonary arterial pressure (13, 13, and 11 mmHg). Furthermore, their clinical and biological characteristics, including the heparin dose and ACT, heparin-ACT response slope, leukocyte and platelet count, baseline complement and eicosanoid levels, arterial blood gases, and duration of cardiopulmonary bypass, were indistinguishable from the patients who did not develop an acute hemodynamic and airway response to protamine.

TxB₂, 6-KETO-PGF_{1α}, PGE₂, AND C5A

After bypass, the plasma level of TxB₂ in arterial blood of the 27 patients in whom TxB₂ was measured was 0.17 ± 0.12 ng per milliliter (x ± S.D., range, 0.04–0.59). In the two responding patients sampled prior to protamine, TxB₂ concentrations were within this range (0.26 and 0.34 ng per ml). All three patients with pulmonary hypertension and increased airway pressure had greatly elevated levels at 1 min (range, 7.4–18.6 ng per ml), whereas TxB₂ was unchanged in the remaining patients (mean, 0.15 ± 0.07 ng per ml). Figure 2 shows the association of thromboxane B₂ levels and changes in mean pulmonary arterial pressure and peak airway pressure. There was no overlap between reactors and non-reactors in either measurement. TxB₂ levels in the reactors were still elevated at 10 min (mean, 5.01 ng per ml; range, 2.78–9.03).

Measurements of C5a anaphylatoxin were performed in the three reactors in addition to nine non-reacting pa-

TABLE I. Hemodynamic Data*

Patient Group†	Variable	Time Relative to Protamine Injection			
		-5 Min	-1 Min	+3 Min	+10 Min
CABG	Heart rate (b/min)	84 ± 2	82 ± 3	83 ± 2	84 ± 2
	Mean arterial pressure (mmHg)	62 ± 3	68 ± 3	69 ± 5	72 ± 5
	Mean pulmonary arterial pressure (mmHg)	15 ± 1	15 ± 1	16 ± 1	13 ± 1
	Central venous pressure (mmHg)	4 ± 1	4 ± 1	4 ± 4	4 ± 1
	Left atrial pressure (mmHg)	6 ± 1	5 ± 1	6 ± 1	4 ± 1
	Cardiac index (1 × m ² /min)	2.6 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
	Systemic vascular resistance index (mmHg × min/1 × m ²)	27 ± 2	28 ± 3	27 ± 3	30 ± 4
	Pulmonary vascular resistance index (mmHg × min/1 × m ²)	3.6 ± 0.3	4.3 ± 0.4	4.0 ± 0.4	3.7 ± 0.4
MVR	Heart rate (b/min)	91 ± 6	89 ± 6	88 ± 6	91 ± 6
	Mean arterial pressure (mmHg)	71 ± 3	73 ± 3	73 ± 4	75 ± 4
	Mean pulmonary arterial pressure (mmHg)	22 ± 2	22 ± 2	21 ± 2	22 ± 2
	Central venous pressure (mmHg)	6 ± 1	6 ± 1	5 ± 1	5 ± 1
	Left atrial pressure (mmHg)	10 ± 1	10 ± 1	10 ± 1	10 ± 1
	Cardiac index (1 × m ² /min)	3.0 ± 0.2	3.0 ± 0.2	2.9 ± 0.2	2.9 ± 0.2
	Systemic vascular resistance index (mmHg × min/1 × m ²)	23 ± 2	24 ± 2	24 ± 3	26 ± 3
	Pulmonary vascular resistance index (mmHg × min/1 × m ²)	4.1 ± 0.4	3.8 ± 0.5	3.9 ± 0.6	4.1 ± 0.5

* Data are means ± S.E.M. values. No statistically significant changes were observed over time.

† CABG: coronary artery bypass graft patients, n = 18; MVR: mitral valve replacement patients, n = 9.

tients. Plasma C5a levels, ranging from undetectable to 2.7 ng per ml (mean, 0.85 ± 1.06 ng per ml) were transiently increased (9.8, 9.9, 10.2 ng per ml) only in the subjects in whom TxB₂ increased (fig. 2). In two responders, C5a had returned to preinjection levels at 10 min. In

the other responder, a secondary rise occurred 5 min after protamine (fig. 1).

Plasma levels of 6-keto-PGF_{1α} before protamine were undetectable, and increased only in one of the reactive patients (fig. 1). Plasma PGE₂ concentrations were un-

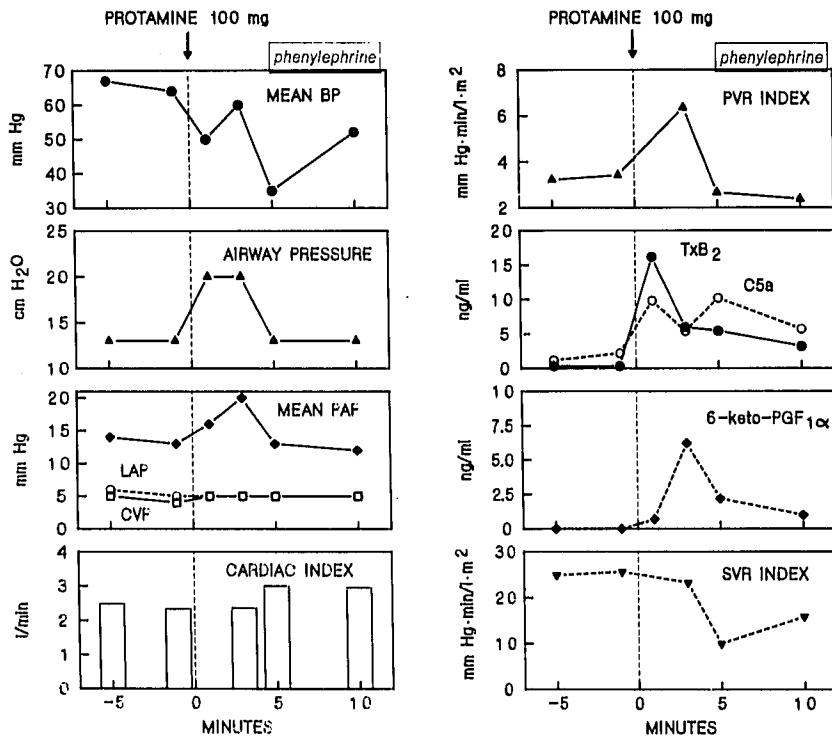


FIG. 1. Hemodynamics, airway pressure, and plasma TxB₂, C5a, and 6-keto-PGF_{1α} in one patient (#14). Systemic hypotension was treated with calcium chloride (500 mg) and a phenylephrine infusion started at 5 min. Note the delay of 6-keto-PGF_{1α} release and its temporal relationship to the secondary decrease of arterial blood pressure.

detectable in all subjects. Measurements were similar in samples drawn from the radial artery and the pulmonary artery, so that no consistent transpulmonary gradient of TxB₂, 6-keto-PGF_{1α}, or C5a was detected.

PLASMA LEVELS OF C3A AND C4A ANAPHYLATOXINS

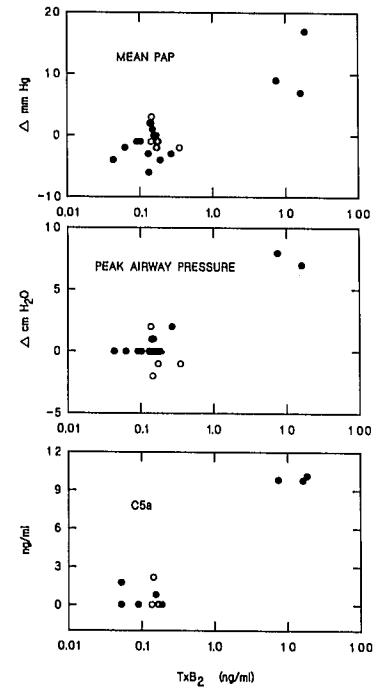
Arterial plasma levels of C3a and C4a increased significantly ($P < 0.0001$) from pre-bypass values to the control pre-protamine values (table 2). Patients who received the 100-mg dose had a significant further increase both of C3a and C4a after protamine. In the first reactor, C3a and C4a concentrations rose moderately with the administration of protamine; whereas, in the two other reactors, C3a and C4a levels were among the highest of the entire patient group (C3a, 3829 and 5519 ng per ml; C4a, 4395 and 7483 ng per ml).

LEUKOCYTE AND PLATELET COUNTS

The 100-mg protamine dose produced an acute leukopenia which was maximal at 1 min, with a mean decrease of $28.9 \pm 21.1\%$ at its nadir (fig. 3, left panel). The degree of arterial leukopenia 1 min after protamine was proportional to the transient transpulmonary leukocyte concentration gradient (fig. 3, right panel), and correlated with the maximum increase of C3a and C4a ($r = -0.71$, $P < 0.001$, and $r = -0.68$, $P < 0.001$).

The 100-mg protamine dose also significantly decreased the platelet count ($-19.3 \pm 14.3\%$ of preinjection values, $P < 0.001$, $n = 18$) at the 10-min sampling period. In contrast to leukocytes, there was no transpulmonary platelet gradient. Red blood cell count and hematocrit did not change during the study period.

FIG. 2. Relationship between plasma TxB₂ levels 1 min after protamine and peak changes in mean pulmonary arterial pressure and peak airway pressure, and absolute plasma C5a levels. Note that TxB₂ levels are shown on a logarithmic scale. Each point represents the data from one patient. Closed circles = 100 mg protamine; open circles = 20 mg protamine. The three patients who reacted are clearly separated from the others. One reactor had no pre-protamine airway pressure recorded.



HISTAMINE

Arterial plasma histamine levels increased slightly though significantly ($P = 0.02$, $n = 27$) with protamine administration, from 0.14 ± 0.12 ng per ml to 0.28 ± 0.24 ng per ml. One patient demonstrated a major elevation of plasma histamine concentration from 0.7 to 10.4 ng per ml in arterial blood and 5.9 ng per ml in mixed venous blood 1 min after a 100-mg dose of protamine, recovering to near baseline levels at 10 min. TxB₂, C5a, 6-keto-

TABLE 2. Plasma Levels of C3a and C4a Anaphylatoxins

Anaphylatoxin Type	Dose of Protamine*	Time Relative to Protamine Injection							F _{ratio} †	P
		Pre-bypass‡	-5 Min	-1 Min	+1 Min	+3 Min	+5 Min	+10 Min		
C3a ng/ml	100 mg	Mean§ 360	1283	1082	1822	1600	1956	2137	2.81	0.02
	Limits¶	286-453	1109-1485	950-1233	1496-2219	1395-1836	1631-2345	1813-2520		
C3a ng/ml	20 mg	Mean§ ± 471	1513	1341	1586	1459	1517	1394	0.09	>0.1
	Limits¶	353-630	1318-1738	1154-1559	1288-1953	1174-1813	1205-1909	1086-1788		
C4a ng/ml	100 mg	Mean§ 574	1160	1181	3573	2650	3273	3405	4.61	<0.001
	Limits¶	479-687	941-1429	985-1417	2629-4856	2058-3413	2492-4298	2707-4290		
C4a ng/ml	20 mg	Mean§ 730	1353	1288	1896	1724	1863	1822	1.09	>0.1
	Limits¶	654-816	1163-1573	1097-1512	1620-2219	1431-2077	1578-2199	1555-2136		

* 100 mg, $n = 19$; 20 mg, $n = 9$.
 † Pre-bypass data not included in this calculation.
 ‡ Pre-bypass levels were significantly different from post-bypass values in all four groups (paired t test).

§ These calculations represent the antilogarithm of mean values subjected to logarithmic transformation.
 ¶ These limits represent 68% confidence intervals for the mean, equivalent to ± 1 S.E.M.

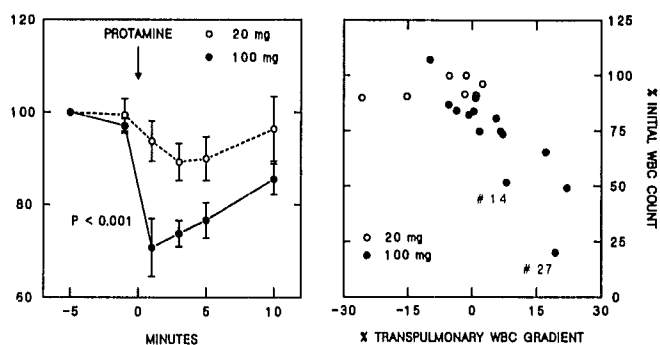


FIG. 3. Arterial white blood cell (WBC) counts and transpulmonary leukosequestration during protamine administration. WBC are expressed as percentage of values 5 min before protamine injection. The 100-mg dose of protamine produced a significant leukopenia ($F = 9.07$, $P < 0.0001$), persisting for 10 min after drug administration. With the 20-mg dose, the WBC decrease was not statistically significant. On the left panel, each point represents the mean (\pm S.E.M.) of observations in 17 patients who received 100 mg (closed circles) and seven patients who received 20 mg (open circles) of protamine. The right panel shows the relationship between arterial WBC count and simultaneous transpulmonary WBC gradient, expressed as percentage of leukocytes measured in mixed venous blood, observed at 1 min after protamine injection. A positive gradient signifies a sequestration; a negative gradient, a release of WBC within the pulmonary circulation. Each point represents individual data from patients receiving either 100 mg (closed circles) or 20 mg (open circles) of protamine. The degree of arterial leukopenia 1 min after protamine was proportional to the transient transpulmonary leukocyte gradient ($r = -0.86$, $P < 0.0001$). Also indicated are the WBC changes of two of the responders (#14 and #27). The data from the third reactive subject are not shown because no pre-protamine blood sample had been obtained. However, this patient had a WBC count of 2300 1 min after injection and of 5500 at 10 min, which strongly suggests that an acute decrease of leukocytes of more than 50% had occurred; in addition, in this patient, a transpulmonary gradient of 600 cells was measured at 1 min, corresponding to a 20.7% of leukocytes sequestered within the lung.

PGF_{1 α} , and PGE₂ were unchanged. This patient immediately became hypotensive from a level of 70–37 mm Hg mean arterial pressure, due to a decrease in systemic vascular resistance. In contrast to the patients with plasma TxB₂ elevations, mean pulmonary arterial pressure and pulmonary vascular resistance index decreased from 25 to 15 mm Hg and 4.4 to 1.7 mm Hg \times min/1 \times m², respectively. Airway pressure was unchanged. Hypotension was treated with epinephrine, and no sequelae were recognized.

Discussion

The most important finding of this study is that patients who respond to protamine neutralization of heparin with pulmonary hypertension and bronchoconstriction demonstrate a different and characteristic mediator profile from those who do not. Arterial TxB₂ and C5a levels were remarkably increased only in patients who manifested this reaction. We have recently documented a con-

sistent cause-and-effect relationship between protamine neutralization of heparin, complement activation, thromboxane release, and pulmonary vasoconstriction in sheep.^{25–27} This implies that the same mediator changes we measured in humans are related to the same adverse physiologic responses we observed in humans.

The major limitation of our study is that we report only three patients (one of whom was not identified prospectively) who responded with pulmonary vaso- and broncho-constriction, despite prospectively studying 48 patients, reflecting the rarity of the response. A recent prospective 1-yr survey in a single institution reported a total of two reactions (only one of which was associated with pulmonary hypertension) attributed to protamine in 1743 cardiac surgical patients.²⁸ We considered it unnecessary and unjustified to expand this series, particularly since the consistency of the mediator profile argues persuasively that C5a and thromboxane are responsible for the observed pulmonary vaso- and broncho-constriction. The fortuitous occurrence of a hypotensive response in a fourth patient who did not demonstrate pulmonary vaso- or broncho-constriction was associated with a different mediator profile, strengthening this line of reasoning.

Thromboxane A₂ is a potent pulmonary vasoconstrictor and bronchoconstrictor. In animal models of acute pulmonary artery hypertension produced by infusion of arachidonate,⁶ endotoxin,⁷ thrombin,⁸ platelet activating factor,⁹ reactive oxygen metabolites,¹⁰ and zymosan-activated plasma,^{4,5} or after institution of partial extracorporeal perfusion,¹¹ there is a concomitant increase of plasma thromboxane B₂, the stable metabolite of TxA₂. Cyclooxygenase blockade or specific inhibition of thromboxane synthesis greatly reduces or completely blocks the increase of TxB₂, as well as the pulmonary vasoconstriction, suggesting that thromboxane is a major agonist of pulmonary hypertension in these models.^{4–11} Furthermore, since infusing pulmonary vasoconstrictors, such as an endoperoxide analogue¹¹ or angiotensin II,²⁹ in animals does not increase plasma TxB₂, we believe rapid thromboxane release causes pulmonary hypertension, rather than resulting from it.

Our plasma TxB₂ levels prior to protamine administration are comparable to those reported after cardiopulmonary bypass.³⁰ However, the elevation occurring immediately following protamine reactions represents the highest plasma levels yet reported in man, and the first to be implicated in a pathophysiologic response in this species. Definition of the dose-response relationship in humans requires further investigation.

Our data suggest that thromboxane is generated when complement activation produces sufficient C5a anaphylatoxin fragments to elevate plasma levels. It has been demonstrated that heparin-protamine complexes cause complement liberation *in vitro*.¹⁶ Whereas transient leu-

kopenia and complement activation as manifested by increased C4a and C3a levels after protamine administration were common, only the three patients with markedly increased plasma levels of C5a had an elevated plasma TxB₂ and bronchoconstriction and pulmonary vasoconstriction. Recently, Kirklin *et al.* measured complement anaphylatoxins C3a, C4a, and C5a in 20 patients before and after protamine infusion following cardiopulmonary bypass.¹⁷ The levels of C3a and C4a they measured were comparable to those in our series. However, they observed neither detectable increases of C5a levels nor acute hemodynamic and airway responses. We are uncertain why our three patients had such elevated plasma levels of C5a. They may have had lower levels of complement pathway inhibitors, altered levels of white blood cell receptors, or fortuitous activation as demonstrated *in vitro*¹⁶ and in experimental animals.³¹

The response we observed in humans resembles closely that reported by others in experimental animals during intravenous zymosan activated complement administration, which causes acute leukopenia, transpulmonary neutrophil sequestration associated with the pulmonary production of thromboxane, pulmonary vasoconstriction, and arterial hypoxemia.^{4,5} Infusion of purified C5a has also been shown to produce the same response³² and to induce the release of prostaglandins, including thromboxane.³³ Although C5a can contract smooth muscles,³⁴ infusion of zymosan activated plasma into sheep pretreated with a cyclooxygenase inhibitor blocks both thromboxane liberation and the pulmonary vasoconstriction and hypoxemia observed in untreated animals.^{4,5} This suggests that thromboxane, rather than C5a, is the primary mediator of pulmonary hypertension and bronchoconstriction after protamine administration.

Prolonged exposure to NPH insulin has been associated with an increased risk of developing a variety of severe protamine reactions.¹⁹ Some responses have been attributed to sensitization to the immunogenic polypeptide protamine, since IgG antibodies directed against protamine have been measured in the sera of NPH insulin-dependent diabetics.²⁰ Though two of our reactors were diabetics, the lack of increase of plasma histamine, PGF_{1α}, or PGE₂, which are released during anaphylactic reactions,¹⁵ suggests a nonimmunological anaphylactoid mechanism, despite the history of protamine administration.

Although the cardiopulmonary reactions to protamine documented in this prospective study were relatively mild, we have previously reported life-threatening pulmonary vasoconstriction producing acute right ventricular failure, left ventricular hypovolemia, and severe systemic hypotension associated with protamine reversal of heparin.¹ The present study elucidates the mediator sequence associated with this adverse unpredictable drug reaction. It

suggests the possibility of avoiding these reactions by administering selective inhibitors of eicosanoid metabolism. Further studies are necessary to define the predisposing factors which lead to this acute reaction.

ADDENDUM

Since submission and review of this paper, we have observed an additional episode of severe pulmonary vasoconstriction with protamine injection requiring reinstitution of cardiopulmonary bypass. The patient is an NPH dependent diabetic with preexisting pulmonary hypertension who had received 10 mg protamine infused slowly over 3 min. His arterial plasma thromboxane B₂ level 3 min after recognition of the response was 7 ng/ml.

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