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shoulder pain, that is not well-controlled with thoracic epidural fentanyl, or fentanyl/bupivacaine continuous infusions. This shoulder pain is variable in intensity and may be severe in many patients. An uncontrolled clinical trial appears to support the early institution of parenteral ketorolac therapy to control these pain symptoms. Future studies are needed to compare the relative efficacy of epidural morphine *versus* fentanyl, with and without ketorolac, on the incidence of shoulder pain following thoracic surgical procedures.

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## High-titer Protamine-specific IgG Antibody Associated with Anaphylaxis: Report of a Case and Quantitative Analysis of Antibody in Vasectomized Men

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PROTAMINE, which is used to reverse the anticoagulating effects of heparin, may cause several adverse re-

actions, including bronchospasm, pulmonary artery hypertension, and systemic hypotension.<sup>1</sup> The mechanisms underlying protamine reactions are not understood completely and appear to be multifactorial.

Several putative high-risk populations for serious protamine reactions have been identified. One such population is vasectomized men.<sup>2-5</sup> Within a year after vasectomy, 50-60% of men develop agglutinating autoantibodies against spermatozoa<sup>3-6</sup> and 22-30% develop autoantibodies against human protamine.<sup>2,3</sup>

Commercial protamine preparations are made from the sperm of salmon and related fish species.<sup>7</sup> Although protamines of different species can be heterogeneous,<sup>8</sup> fish and human protamines are similar and immunologic cross-reactivity is possible.<sup>4</sup>

We have shown previously that, in protamine-insulin dependent diabetic persons, the presence of either protamine-specific IgE or IgG is a significant risk factor for severe, life-threatening reactions when intravenous

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protamine is administered.<sup>9</sup> We now describe a man with a prior vasectomy who had two severe life-threatening reactions after intravenous administration of protamine. In addition, we determined the prevalence and quantity of serum protamine IgG and IgE antibodies in a population of vasectomized men.

### Case Report

A 63-yr-old man with a history of severe mitral regurgitation was taken to the operating room for mitral valve repair. His past medical history was significant for a vasectomy. He had no history of diabetes mellitus or fish allergy. His only previous exposure to protamine was 45 mg given intravenously at cardiac catheterization 6 weeks prior to surgery.

Heparin (22,000 units) was given to the patient before initiation of cardiopulmonary bypass. A quadrangular resection and repair of the posterior leaflet of his mitral valve and insertion of a Carpentier #34 anuloplasty ring were performed. Following successful completion of the surgical repair, the patient was separated easily from bypass, and protamine was administered to neutralize the heparin. Within 5 min after the slow infusion of 50 mg protamine, severe systemic hypotension and pulmonary artery hypertension developed in the patient. His systolic blood pressure decreased from 95 to 40 mmHg.

The patient was treated immediately with intravenous calcium chloride, phenylephrine, epinephrine, and intravenous fluids (lactated Ringer's solution), which promptly restored his blood pressure. His postoperative course was remarkable for congestive heart failure and unsustained ventricular tachycardia. His arrhythmias were treated initially with medications; however, on the fifth postoperative day, the patient suffered a cardiac arrest secondary to sustained ventricular tachycardia, which was treated successfully with cardioversion and medications. Two weeks later, he returned for electrophysiologic studies for recurrent ventricular tachycardia.

Following the electrophysiologic studies, protamine was administered to reverse the heparin anticoagulation. Several minutes after the protamine infusion began, hypotension and bradycardia developed in the patient. His hemodynamics were restored promptly after intravenous fluids, antihistamines, and corticosteroids. He returned to his room in stable condition and was discharged from the hospital several days later.

### Methods

#### Subjects

Serum from the patient was drawn at the time of his reactions and 3 months later when he returned to the clinic for follow-up care. Following institutional approval, sera were obtained from 55 vasectomized men ranging in age from 21 to 45 yr. The serum samples were drawn from 8 months to 25 yr after vasectomy. None of the patients had any known prior exposure to protamine.

The control group consisted of 50 age-matched men with no history of a vasectomy, infertility, or prior exposure to protamine.

#### Protamine-Specific IgG Assay

Protamine-specific IgG antibody was measured using an agarose-based staphylococcal protein A solid-phase radioimmunoassay. Sera were preincubated with 5% (v/v) Sepharose CL-4B beads (Pharmacia, Piscataway, NJ) overnight to remove naturally occurring anti-agarose IgG.<sup>10</sup> Sera were analyzed in dilutions of 1:40 or greater (made in phosphate-buffered saline containing bovine serum albumin and Tween 20), and all dilutions beyond 1:40 were made in 1:40 normal human serum to maintain the quantity of total IgG constant.<sup>11</sup> All agarose-absorbed samples then were analyzed in triplicate. Briefly, sera were incubated with the protamine-agarose sorbent and orbitally rotated for 4 h at room temperature. After washing, the antibody-coated particles were incubated overnight with I<sup>125</sup> protein A. After a second wash, the bound radioactivity was quantitated by gamma spectroscopy and bound results (in counts per minute) were interpolated from a dilution curve of the reference serum with known IgG antibody content. This reference curve was mathematically fit by a three-parameter cubic spline function, as previously described.<sup>12</sup> The analytical sensitivity of the protamine-specific IgG assay was 40 ng/ml.

#### Protamine-Specific IgE Assay

Protamine-specific IgE antibody was measured in a manner similar to the assay described for IgG antibody, except for the following modifications: (1) no preabsorption with agarose beads was performed, (2) sera were analyzed at different dilutions (1:1 and 1:2), and (3) I<sup>125</sup> radiolabeled rabbit antihuman IgE was substituted for I<sup>125</sup> protein A.

In both assays, inhibition of protamine-specific IgG and IgE binding to the protamine sorbent was evaluated by a 1-h preincubation of sera with soluble protamine sulfate (1.4 mg/ml) before addition to the protamine-agarose sorbent. The analytical sensitivity of the protamine-specific IgE assay was 0.1 ng/ml.

#### Tryptase and Complement

The patient's sera also were evaluated for tryptase by the pharmacia test (Pharmacia, Uppsala, Sweden) and complement C<sub>3</sub> and C<sub>4</sub> by standard radioimmunodiffusion.

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*Expression and Analysis of Data*

The results of the direct-binding studies measuring protamine-specific IgG and IgE antibodies were quantitated in mass units and 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 the inhibition studies measuring protamine-specific antibodies were expressed as the percentage of inhibition, calculated using the following formula: percent inhibition =  $1 - (\text{antibody concentration with inhibition} / \text{antibody concentration with buffer}) \times 100\%$ . Positive serum samples were defined as those with a count per minute  $\geq 2.5$  times that of a negative serum sample and inhibitable  $\geq 50\%$  by soluble protamine. Data were analyzed by chi-square tests.

**Results**

At the time of his first protamine reaction, the patient had a protamine-specific IgG concentration of 53  $\mu\text{g}/\text{ml}$ . Following his second reaction 1 week later, his protamine-specific IgG concentration had increased to 677  $\mu\text{g}/\text{ml}$ . Three months later, his serum concentrations had decreased to 79  $\mu\text{g}/\text{ml}$ . Soluble protamine competitively inhibited the binding of protamine-specific IgG antibodies to the protamine sorbent greater than 50% in all three serum samples. The patient had no detectable protamine-specific IgE antibody.

The patient's trypsin and complement concentrations were within normal limits except for a low  $C_4$  (0.09 g/L, normal 0.16–0.45 g/L) found in the patient's serum after his first reaction to protamine. This low value may indicate the consumption of complement during his reaction.

Of the 55 vasectomized men in the study, 16 (29%) had protamine-specific IgG antibody. In contrast, none of the 50 control patients had protamine-specific IgG antibody. Soluble protamine competitively inhibited the binding of protamine-specific IgG to the protamine sorbent in all 16 of the positive serum samples (table 1). The specificity of the inhibition was confirmed by the inability of soluble protamine to inhibit the direct binding of ragweed-specific IgG to a ragweed agarose sorbent (mean inhibition = 0,  $n = 4$ , data not shown). Neither the study population nor the control group had detectable concentrations of protamine-specific IgE antibody.

Thirty-two of the 55 vasectomized men (58%) had sperm-specific antibody. Nine of the 16 patients (56%) who were positive for protamine-specific IgG had

**Table 1. Serum Protamine-specific IgG in 16 Vasectomized Men**

Patient No.	Protamine-specific IgG ( $\mu\text{g}/\text{ml}$ )	% Inhibition with Soluble Protamine
1	6.8	100
2	1.5	99
3	1.7	93
4	4.0	100
5	0.9	95
6	4.8	100
7	0.8	99
8	0.9	98
9	10.9	78
10	8.3	69
11	5.1	82
12	6.4	67
13	10.0	77
14	7.2	71
15	5.5	82
16	4.1	100

sperm-specific antibody. No association was found between the presence of sperm-specific and protamine-specific antibodies.

**Discussion**

Protamine neutralization of heparin after cardiopulmonary bypass, cardiac catheterization, vascular surgery, dialysis, or plasmapheresis had been associated with rare and unpredictable adverse reactions. Acute reactions attributed to protamine range from a mild rash and urticaria to severe bronchospasm, pulmonary artery hypertension, and profound shock.<sup>1</sup> Several mechanisms have been postulated as the etiology of these reactions. These mechanisms include complement activation, either through protamine-heparin complexes<sup>13–15</sup> or through interactions of protamine-specific IgG<sup>16</sup>; thromboxane generation<sup>17–19</sup>; inhibition of carboxypeptidase N<sup>20</sup>; and immediate hypersensitivity reactions triggered by protamine-specific IgE antibodies.<sup>9</sup> More than one mechanism is likely contributing to these complex reactions.

Several high-risk groups with an increased frequency of reaction to protamine have been identified. Diabetic patients receiving protamine-containing insulin preparations have been shown to have a 40- to 50-fold increased risk for life-threatening reactions when given intravenous protamine.<sup>21,22</sup> Patients with a history of vasectomy have a postulated but heretofore undemon-

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strated increased risk of serious protamine reactions.<sup>2-6,23</sup>

Samuel has shown that certain fish and human protamines share common antigenic determinants. Using micro-complement fixation studies, he demonstrated immunologic cross-reactivity between human and salmon protamines.<sup>4</sup> These antibodies develop progressively after vasectomy.<sup>4</sup> Antibody formation normally is prevented by the blood-testes barrier. When vasectomy blocks the normal ejaculatory pathways and the blood-testes barrier is disrupted, systemic absorption of sperm occurs, which can result in the formation of antibodies to these "new" antigens. Tung, using indirect immunofluorescence techniques, found that antibodies to various components of spermatozoa develop in vasectomized men. In a related study also using indirect immunofluorescence, Samuel *et al.* found that 17 of 78 vasectomized men (22%) developed antibodies to human protamine within a year after vasectomy.<sup>3</sup> In all studies, antibodies directed against protamine were not found in sera from men who did not have a vasectomy.

Similarly, in our study, protamine-specific IgG antibody developed in 29% of vasectomized men, as compared to 0 in nonvasectomized controls. The presence of protamine antibody in vasectomized men and the immunologic cross-reactivity between human and salmon protamine underscore the potential for immunologic reactions to protamine. However, the concentrations of protamine-specific IgG in the vasectomized men were much lower than in the patient with anaphylaxis.

We have described a case of anaphylaxis from intravenous protamine in a vasectomized man whose only prior exposure to protamine was at cardiac catheterization. One other case of a protamine reaction in a vasectomized patient has been reported.<sup>5</sup> In that reaction, pruritus and hives developed within 5 min after administration of intravenous protamine during a cardiac catheterization. The patient had no wheezing or change in blood pressure, and his symptoms resolved with intravenous diphenhydramine hydrochloride. The presence of protamine-specific antibody was demonstrated in the patient's serum using a tray agglutination test and antiprotamine immunofluorescence. Similarly, we found high concentrations of protamine-specific IgG antibody in our patient's sera using a solid-phase radioimmunoassay.

Our data suggest that IgG antibodies to protamine develop in about 29% of vasectomized men, whereas

IgE antibodies do not. In our previous study, high titers of protamine-specific IgG were a significant risk factor for protamine reactions even in the absence of IgE.<sup>9</sup> Adverse reactions in patients with IgG antibody could be mediated by complement activation and anaphylatoxin generation by the interaction between protamine and protamine-specific IgG.<sup>12-15</sup>

Our study shows that vasectomized men have an increased incidence of protamine-specific IgG antibody in their sera compared to controls. This case report demonstrates that vasectomized men with high titers of protamine-specific IgG antibody may be at increased risk for life-threatening reactions to protamine.

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## Acute Right Ventricular Failure after Pulmonary Hypertensive Responses to Airway Instrumentation: Effect of Fentanyl Dose

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ACUTE right ventricular failure and hemodynamic collapse are potential threats in patients with severe pulmonary hypertension undergoing general anesthesia. Pulmonary hypertension and pulmonary hypertensive crises are a known risk factor for mortality in children with congenital heart disease.<sup>1-5</sup> In these patients, pulmonary hypertensive responses as well as systemic hypertensive responses need to be controlled to avoid

acute increases in right ventricular afterload, subsequent right ventricular dysfunction, and possible acute right ventricular failure secondary to ischemia.<sup>6,7</sup>

Low-dose fentanyl (8  $\mu$ g/kg) in combination with thiopental (3 mg/kg) for induction of anesthesia has been shown to blunt circulatory responses to tracheal intubation,<sup>8</sup> but responses of the pulmonary circulation to intubation after this regimen have not been reported. Several studies document the effectiveness of higher-dose fentanyl (25-100  $\mu$ g/kg) in providing both systemic and pulmonary hemodynamic stability in children undergoing cardiac surgery and preventing pulmonary hypertensive responses to airway stimulation.<sup>9,10</sup> However, little is known about the minimal dose of fentanyl or other opioids required to blunt pulmonary vascular reactivity.

We present a case of documented acute right ventricular failure secondary to a pulmonary hypertensive crisis provoked by upper airway instrumentation. This

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