Protamine-Induced Histamine Release in Human Skin Mast Cells

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Rapid intravenous (iv) infusion of protamine sulfate is associated with hypotension in humans. A possible mechanism for this hypotension is the release of inflammatory mediators, including histamine, from tissue mast cells lining the blood vessels. To determine whether protamine caused nonimmunologic release of histamine, histamine release from dispersed human skin mast cells exposed to protamine sulfate was measured. Skin from seven adult patients was washed, chopped into small tissue fragments, and incubated with collagenase, hyaluronidase, and DNAase. Dispersed mast cells were harvested after 12 h of short-term tissue culture, washed, and challenged with protamine sulfate. Histamine release was measured using an automated histamine analyzer and expressed as a per cent of total released histamine measured minus the spontaneous histamine release. Spontaneous histamine release averaged 6 ± 1%. Protamine produced dose-related histamine release. At a concentration of 3 × 10⁻⁵ M, protamine sulfate released 14 ± 2% (P < 0.05), which significantly differed from spontaneous release. This study demonstrates that protamine sulfate causes nonimmunologic histamine release in dispersed human skin mast cells. However, histamine release occurred only at concentrations much greater than those used in clinical practice. Thus, these data do not support the hypothesis that nonimmunologic histamine release is a likely mechanism for protamine-induced hypotension in vivo. (Key words: Histamine release. Mast cells. Protamine.)

Protamine sulfate is derived from salmon melt with a molecular weight ranging from 4,500 to 10,000. The reversal of heparin anticoagulation and retardation of insulin absorption are the two current medicinal uses of protamine. Adverse reactions to protamine are well known and range from transient hypotension to cardiovascular collapse and death. These adverse reactions include urticaria, bronchospasm, and pulmonary hypertension. Diabetic patients receiving daily subcutaneous injections of insulin-containing protamine appear to have a 40- to 50-fold increased risk of life-threatening reactions when given protamine intravenously. An association between protamine IgE and IgG antibodies and life-threatening reactions to intravenous (iv) protamine reactions has been demonstrated.

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The rapid administration of protamine has been more commonly associated with hypotension that does not appear to be either IgE- or IgG-mediated. Hypotension and increased histamine concentrations have been reported in association with the rapid infusion of vancomycin, morphine, and d-tubocurarine (d-Tc). The hypotension associated with rapid protamine administration can be partially prevented by histamine receptor blockade in patients following cardiopulmonary bypass. It is possible, therefore, that the non-specific release of inflammatory mediators, specifically histamine, may be one explanation for the hypotension associated with rapid protamine infusion. The objective of this study was to test the hypothesis that protamine sulfate in concentrations used clinically produces histamine release from dispersed human skin mast cells.

Materials and Methods

This study was exempted from institutional review. The methods have been previously described and are summarized here. Human skin was obtained from seven adult patients undergoing a variety of surgical procedures, most often face-lifts and reduction mammoplasties. The skin was immediately placed in calcium- and magnesium-free Hank's balanced salt solution (CMF-HBSS) (Gibco, Grand Island, New York) and transported fresh to the laboratory. The skin was then scored, chopped into small fragments (1–2 mm), and washed twice with CMF-HBSS. Skin fragments were then incubated with collagenase (20 mg/g wet weight) (Gibco), hyaluronidase (4 mg/g wet weight) (Sigma, St. Louis, Missouri), and DNAase 1,000 units (Calbiochem, Behring Diagnostics, La Jolla, California) at 37°C for 3 h with constant stirring. The digested tissue fragments were washed with 3.5% bovine serum albumin (Sigma) three times and filtered through a Nitex cloth. The resulting cell pellet was resuspended, stained with Mota stain, and counted. Cell viability was ascertained by trypan blue exclusion staining. The dispersed mast cells were then suspended in 25 ml of RPMI 1640 (Gibco), 1% gentamicin (Gibco), and 5% fetal calf

serum and placed in short-term culture for 12 h in humidified 95% air and 5% CO₂ at 37°C. After 12 h the dispersed skin mast cells were harvested, washed with PAG (25 mM piperazine-N,N′bis[2-ethane-sulfonic acid]) [PIPEC buffer; Sigma] 110 mM NaCl, 6 mM KCl, 0.1% dextrose) three times and brought to a final concentration of 3 \times 10^6 mast cells/ml for challenge with protamine sulfate (Calbiochem, Behring Diagnostics). The calcium ionophore A23187 (Calbiochem, Behring Diagnostics), a known nonspecific histamine releaser, was used as a positive control in a concentration of 1 ng/ml. Histamine release was measured using an automated histamine analyzer.⁹ The total histamine release was determined by high-frequency sonication for 10 s. The percent histamine release was equal to the measured histamine release minus the spontaneous histamine release divided by the total histamine release and expressed as a percentage. If spontaneous histamine release was greater than 15% of the total histamine release, then the mast cells were considered damaged by the digestion process and discarded. The data were analyzed by two-way analysis of variance (ANOVA) and expressed as the mean ± SEM. A P value of less than 0.05 was considered significant. The significant difference between paired groups was then tested by the least significance difference method.

**Results**

Spontaneous histamine release averaged 6 ± 1% in mast cells that were estimated to be 75–85% viable by trypan blue exclusion staining. Protamine sulfate caused histamine release in a dose-related manner in doses of protamine ranging from 1 \times 10^{-5} M to 3 \times 10^{-3} M (Fig. 1), which reached statistical significance only at a concentration of 3 \times 10^{-3} M. A23187, the positive control (10^{-6} M), caused reliable and reproducible histamine release (70 ± 10%) (P < 0.05).

**Discussion**

Commercially available protamine is a highly alkaline, polycationic compound derived from salmon melt. Adverse reactions to the i.v. infusion of protamine are well known and are of three main types; 1) transient hypotension associated with the rapid infusion of protamine sulfate;¹ ² ³ ⁴ ⁵ ⁶ ⁷ ⁸ ⁹ ¹⁰ ¹¹ ¹² ¹³ ¹⁴ ¹⁵ ¹⁶ ¹⁷ ¹⁸ ¹⁹ ²⁰ ²¹ ²² ²³ ²⁴ These include inhibition of cardiac performance,¹¹ ¹² ¹³ ¹⁴ ¹⁵ increases in prostaglandins,¹⁵ decreases in ionized calcium,¹⁶ ¹⁷ activation of the complement cascade,¹⁸ ¹⁹ ²⁰ ²¹ and generation of anaphylatoxins and thromboxane.²² ²³ The release of cellular mediators, especially histamine, as a result of mast cell degranulation has also been suggested as a possible mechanism for transient hypotension associated with the rapid infusion of protamine. Histamine is known to produce hypotension in humans²² by direct vasoconstrictor effects on peripheral blood vessels,²⁵ thereby decreasing systemic vascular resistance.²⁴ These observations would be consistent with the hemodynamic alterations observed with the rapid infusion of protamine.¹ Levy et al.,²⁵ however, were unable to prospectively predict by adding protamine to preoperative blood samples and measuring serum histamine concentrations which patients would be at risk for adverse protamine reactions. More recently, these same investigators were unable to demonstrate protamine-induced nonimmunologic histamine release in specimens of chopped human lung tissue.²⁶ With several different
functionally distinct mast cell populations well described in the literature, we decided to use dispersed human skin mast cells as a model because they are functionally similar to the mast cells lining the blood vessels (Schwartz L: personal communication, May 1988).

Our results clearly demonstrate that protamine sulfate causes dose-related histamine release in dispersed human skin mast cells in vitro. However, this dose-related, non-specific, nonimmunologic histamine release was only significantly different from the negative controls at concentrations of protamine at least 200 times greater than the maximum concentrations ever seen in clinical practice. These data do not support the hypothesis that nonimmunologic histamine release caused by the degranulation of connective tissue mast cells is a likely mechanism that explains the transient hypotension associated with the rapid infusion of protamine sulfate.

References