Protamine and Protamine Reactions

Kyung W. Park, MD

First discovered in 1868, protamine is a polycationic, alkaline protein molecule, made up of two-thirds arginine residues and one-third other amino acids. Heads of the sperm of the salmonidae or clupeidae family as well as man are rich in protamine, and salmon milt is the commercial source of protamine. Clinical uses of protamine include the neutralization of heparin used during cardiac or vascular surgery, cardiac catheterization, dialysis and phoresis, and the retardation of insulin absorption in intermediate- and long-acting insulin preparations. Because protamine is positively charged, it forms a stable complex with the negatively charged heparin, a heterogeneous compound with alternating residues of iduronic acid and glucosamine; iduronic acid contains 2 sulfate groups and is one of the strongest naturally occurring acids. The protamine–heparin complex is devoid of anticoagulant activities. Protamine-containing insulin preparations are neutral protamine Hagedorn (NPH) insulin and protamine zinc insulin (PZI).[1,2] Each unit of NPH insulin contains 3 to 6 μg of protamine, and each unit of PZI has 10 to 15 μg of protamine.

■ Protamine Reactions

Types of Protamine Reactions

Hypotension From Histamine Release As with other basic molecules such as D-tubocurarine, morphine, and meperidine, rapid administration of protamine will lead to systemic hypotension.[3] This is due to the release of histamine from mast cells. The reaction is more likely to occur with rapid injection of protamine. Although the distributor's package insert recommends that protamine be administered no more quickly than 5 mg/min, several studies have shown that patients will tolerate considerably faster rates (heparin neutralizing dose in 5–10 minutes) without significant hemodynamic instability.[4–7] Systemic hypotension is mostly

from a decrease in afterload with minimal changes in preload or contractility of the heart. But the hypotensive response will be less likely to be tolerated by a hypovolemic patient[8] or a patient with poor left ventricular function who is not able to mount a compensatory increase in cardiac output to maintain blood pressure. Earlier interest in left atrial injection of protamine has faded with lack of demonstration of significant benefit from it.

Anaphylactic Reaction Allergy is a hypersensitivity to a specific substance and may result in 4 different types of reactions, which are immediate hypersensitivity, cytotoxic reactions, immune complex disease, and delayed hypersensitivity. Immediate hypersensitivity involves the release of vasoactive mediators from mast cells resulting from contact of the antigenic substance with antibodies adherent to the cells. In a cytotoxic reaction, circulating antibodies (usually immunoglobulin [IgG] other than IgG_4 subclass) react with antigens on the cell surface, leading to lysis of the cells (e.g., hemolytic transfusion reaction). An immune complex is formed from antigens and antibodies interacting freely in blood and may then be deposited in tissues, leading to pathology (eg, Goodpasture syndrome). Delayed hypersensitivity is mediated by T cells sensitized by an antigen (Ag) (e.g., poison ivy contact dermatitis).

When an immediate hypersensitivity reaction is accompanied by cardiovascular collapse, it is termed anaphylaxis.[9] Anaphylactic reactions are mediated by IgE and IgG_4 immunoglobulins.[3] Symptoms occur within seconds to minutes after exposure to the Ag and include systemic hypotension, bronchospasm, and skin and mucous membrane reactions. Upon first exposure to the Ag, the Ag is presented by macrophages to the Ag-specific B lymphocytes, in the presence of lymphokines secreted by T helper cells. Some B cells differentiate into antibody (Ab)-secreting plasma cells, whereas other B cells differentiate into memory cells. The reaction on the first exposure tends to be mild. Upon re-exposure to the Ag, the memory cells amount a swift immunologic response. When the Ag binds IgE on the surface of the mast cells and basophils, these cells degranulate and release inflammatory mediators such as histamine, leukotrienes, cytokines, proteases, arachidonic acid, platelet-activating factors, and enzymatic mediators, which together produce the clinical manifestations. Binding of the Ag to the cell-bound IgG Ab leads to complement activation by the classic pathway. The Ag–Ab complex binds C1, setting off a cascade of reactions. Activated C1s mediates the assembly of C4b2a from C4 and C2, and C4b2a in turn cleaves C3 into C3a and C3b. The complex C4b2a3b then cleaves C5. Subsequent reactions lead to the formation of the C5b-9 membrane attack complex. C3a, C4a, and C5a are anaphylatoxins. C3a causes mast cell degranulation, smooth muscle contraction, and increased capillary permeability. C4a and C5a mediate inflammation.

Protamine may act as an Ag that initiates an anaphylactic reaction.

Diabetic patients who take daily subcutaneous injections of protamine-containing insulin preparations may be at an increased risk of life-threatening reactions to protamine.[10,11] In such patients, the presence of antiprotamine IgE and IgG antibodies is a significant risk factor for acute protamine reactions, with a relative risk factor of 95 and 38, respectively,[12] demonstrating that at least some of the life-threatening protamine reactions may be IgE- and IgG-mediated immediate hypersensitivity reactions.

Anaphylactoid Reactions An anaphylactoid reaction is a life-threatening response of vital organs to a substance and may encompass both IgE-mediated immediate hypersensitivity reactions (anaphylaxis) and non–IgE-mediated reactions. Non–IgE-mediated anaphylactoid reactions to protamine administration may occur through complement activation by antiprotamine IgG or by protamine–heparin complexes. Weiss et al reported that in nondiabetic patients, the presence of antiprotamine IgG has a relative risk of 25 for a life-threatening protamine reactions[12]; in such patients, no one was seen to have antiprotamine IgE. These patients may have developed antiprotamine IgG through prior exposure to protamine during vascular surgery or cardiac catheterization. It may be that development of IgE depends on the dose and frequency of exposure. Protamine–heparin complexes have been shown both in vitro[13] and in vivo[14,15] to activate the complement system via the classic pathway, generating anaphylatoxins C3a, C4a, and C5a. Furthermore, protamine may interfere with the action of plasma carboxypeptidase N, which cleaves the anaphylatoxins to less active metabolites.[16]

Catastrophic Pulmonary Hypertension Lowenstein et al reported 5 patients who experienced precipitous pulmonary hypertension, right ventricular dysfunction, and elevation of right atrial pressure as well as systemic hypotension, following protamine reversal of heparinization.[17] This was in contrast to a classic anaphylactoid reaction, in which pulmonary, right atrial, and left atrial pressures would all be low. The patients were treated with epinephrine and/or calcium with hemodynamic improvement. Later, Lowenstein's group prospectively followed 48 adults undergoing cardiac surgery with use of cardiopulmonary bypass and found 2 patients who had acute bronchospasm, pulmonary hypertension, and systemic hypotension within 1 to 3 minutes of protamine administration.[18] In these patients, there was a large increase in the plasma levels of C5a and thromboxane B_2, but no significant change in histamine. In the rest of the patients, there was no significant change in C5a and thromboxane B_2, the stable metabolite of thromboxane A_2. They theorized that complement-mediated generation of thromboxane A_2 was responsible for the clinical manifestations.

In a pig model, Conzen et al showed that pulmonary hypertension following protamine neutralization of heparin may be prevented by pretreatment with either the cyclo-oxygenase inhibitor indomethacin or a

thromboxane A_2 receptor antagonist,[19] demonstrating the central importance of thromboxane A_2 in this reaction. The reaction was not seen if protamine alone was given without prior heparin, and it might be that the heparin–protamine complex induces complement activation, which in turn leads to the prostanoid production. Administration of protamine is also accompanied by a decrease in platelets and leukocytes, which are trapped in the pulmonary circulation. Although it has been speculated that the trapped platelets and leukocytes might be associated with complement activation and thromboxane generation,[3] pretreatment with indomethacin blocked thromboxane generation and hemodynamic changes, without an effect on the platelet or leukocyte count.[19]

Delayed Noncardiogenic Pulmonary Edema Fulminant noncardiogenic pulmonary edema has been reported 15 minutes to more than an hour after administration of protamine.[3] There is massive pulmonary capillary leak, leading to decreased pulmonary compliance, wheezing, and pulmonary edema. There may also be systemic capillary leak, leading to anasarca. Loss of fluid from the vascular space lowers cardiac filling pressures, cardiac output, and blood pressure. Because of the delayed nature of this syndrome, it has not been possible to pinpoint the blame on protamine administration. Another common finding in the case reports has been transfusion of fresh-frozen plasma and a phenomenon like the transfusion-related acute lung injury may have been at play.

■ Risk Factors for Protamine Reactions

Commercial protamine is prepared from sperm of salmon or related species, and a true (vertebrate) fish allergy has been alleged to be a risk factor for protamine reactions. A case report of a man allergic to fish who had a catastrophic cardiovascular collapse after protamine administration has been published.[20] In this patient, radioallergosorbent test (RAST) against codfish allergen was positive and enzyme-linked immunosorbent assay (ELISA) demonstrated high titers of IgG, IgM, and IgE against protamine sulfate. On the other hand, Levy et al prospectively followed 6 patients with a history of true fish allergy out of a group of 4796 cardiac surgical patients and found that none of the 6 had an adverse reaction to protamine.[21] Because shellfish and vertebrate fish are phylogenetically distinct, shellfish allergy does not add to the risk of an adverse protamine reaction.

Nonvasectomized men have a "blood-testes" barrier that sequesters sperm from the remainder of the body. A vasectomy occludes the normal ejaculatory path and allows sperm to be absorbed systemically and possibly stimulate Ab production. In a recent report, 35% of 55 vasectomized men had significant serum titers of antiprotamine IgG, compared with 0% of 50 age-matched controls.[22] Earlier reports indicated that within a year of

vasectomy, more than 50% of men develop agglutinating autoantibodies against sperm, and 22% to 30% develop autoantibodies against protamine.[23–25] Because similarities exist between the protamines of fish and human sperm, cross-reactivity is possible, but this remains to be demonstrated. In a prospective follow-up, Levy et al found no difference in the incidence of protamine reactions between the vasectomized men (no reaction in 16 patients) and the general population without any predisposing conditions.[21]

Antibodies to protamine are common in diabetic patients taking protamine-containing insulin preparations and the likelihood of antiprotamine IgG increases with duration of use (38% after 1 year to 91% after more than 20 years).[26] Using ELISA, Sharath et al showed that 53% of diabetic patients on NPH had IgE to protamine, whereas none of the patients with diabetes not on NPH or nondiabetics had the antibody.[27] In a retrospective review of 3245 cardiac surgical patients, the incidence of protamine reactions was 1 out of 160 or 0.6% in diabetic patients on NPH insulin and 2 out of 3085 or 0.06% in other patients. The difference was suggestive, but not statistically significant ($P > 0.15$). Likewise, patients with prior exposure to protamine in a clinical setting such as vascular surgery or cardiac catheterization may be at a theoretically increased risk of an adverse reaction to protamine, but this risk is yet to be documented.

Studies that examine risk factors for protamine reactions are fraught with the difficulty of having to sort out the different types of protamine reactions. Retrospective reviews may not yield all the data necessary to establish the diagnosis of a protamine reaction such as the result of an immunologic assay, serum levels of complements or thromboxanes, or the detailed chronological documentation of administration of protamine and subsequent hemodynamic course. Even then, a risk factor such as prior exposure may be a risk factor for certain types of reactions such as immediate hypersensitivity (anaphylaxis), but not others (heparin–protamine complex-mediated activation of the complement system and thromboxane production). Additionally, the incidence of adverse reactions to protamine is quite low, making the task of demonstrating a significant increase in reactions with a risk factor quite a daunting task. At present, it appears that any of the alleged risk factors do not lead to a clinically significant increase in the incidence of adverse protamine reactions, so that the use of protamine is not considered contraindicated in patients with one or more of the "risk factors."

■ Diagnosis of Protamine Allergy

Intradermal skin tests to protamine are performed by injecting 0.01 to 0.02 mL of a 1 μg/mL solution subcutaneously and tests for IgE-mediated hypersensitivity.[3] Horrow et al showed that the criterion for a positive test

is optimized by requiring ≥8 mm induration at 10 minutes; with that criterion, the sensitivity is high at 91% and the specificity is 78%.[28] Because of a relatively high false-positive rate (22%), a positive skin test is not useful in screening patients "at risk." There is also a risk of anaphylaxis to the small amount of antigen injected.

The agarose-based RAST measures antiprotamine IgE antibodies. The Ag is coupled to a solid phase and detects IgE in vitro, using radiolabeled anti-IgE. The test detects minute quantities of antibodies and is highly specific. However, it is expensive and may not be readily available.

In ELISA, anti-IgE, tagged with an enzyme that catalyzes a photochemical reaction, is used to detect small quantities of IgE. Although the manufacturer of the ELISA kit claims a false-positive rate of 6%, a recent study found the rate to be much higher at 46%.[28] Either number would be much greater than the true-positive rate in the population, which is probably ≤1%. In addition, ELISA is expensive and may not be readily available in all centers.

Serum tryptase is an indicator of mast cell involvement in an anaphylactoid reaction.[29] It is released during an anaphylactoid reaction and may be detected for more than 1 hour after the reaction. An elevation in serum tryptase has been reported in a diabetic patient who had an adverse reaction to protamine.[30]

■ Administration of Protamine in Cardiac Surgery

Commonly, a fixed dose of 1.0 to 1.3 mg of protamine for 1 mg or 100 units of heparin is used to neutralize heparin. This dose does not account for heparin elimination and can result in excess circulating protamine. Alternatively, the protamine dose may be calculated based on a heparin activity half-life of 90 minutes; eg, if protamine is given 90 minutes after an initial heparin dose of 400 mg, only 200 mg of protamine would be used before any further dosing is considered based on measurement of the activated clotting time (ACT).

Just as incomplete reversal of heparinization may lead to excessive bleeding, excessive protamine can have deleterious effects on the coagulation system. An overdose of protamine (2 mg of protamine for every mg of heparin) decreases both platelet number and function in dogs.[31] In cardiac surgical patients, protamine: heparin ratios of more than 1.3:1 were associated with alteration of ADP-induced platelet aggregation and prolongation of the ACT.[32] Free protamine may also precipitate fibrinogen[33] and reduce the procoagulant effect of thrombin.[34] Platelet dysfunction associated with excess protamine may prolong the ACT.[32] Thus, the finding of a prolonged ACT after protamine administration could indicate either incomplete heparin neutralization or excess protamine and

may thus be a source of confusion. Visual inspection of the operative field will not aid in distinguishing between the 2 possibilities. If the ACT is prolonged following the initial protamine dosing, then thrombin time, heparinase-ACT, and low-level heparin-protamine titration are methods that can help to determine whether excess heparin is present. The thromboelastogram, platelet count, and fibrinogen levels may also be measured to help manage patients with persistent bleeding after complete neutralization of heparin.

Some adverse reactions to protamine such as histamine-induced systemic hypotension may be related to the speed of administration of the medication, and the distributor's recommendation is not to give it any faster than 5 mg/min. In sheep, the speed of heparin neutralization with protamine was associated with the magnitude of thromboxane-mediated pulmonary hypertension and systemic hypotension.[35] However, strict adherence to the distributor's regimen of ≤5 mg/min may often take more than an hour to administer protamine after termination of CPB and may not be practical. Several clinical studies[4–7] have shown that patients tolerate faster administration of protamine (heparin neutralizing dose of protamine in 5–10 minutes). Interestingly, administration of heparin-neutralizing dose of protamine over 5 minutes versus 30 minutes did not result in any significant difference in the amount of bleeding or return of the ACT to the baseline.[36]

■ Management of the Patient with Known Hypersensitivity to Protamine

Treatment of an acute anaphylactoid reaction to protamine consists of supporting the affected organ systems and possibly reducing the release of vasoactive and bronchoactive mediators.[9] Diphenhydramine 50 mg intravenously may help with the cutaneous manifestations of anaphylaxis and counteract gastrointestinal, uterine, and smooth muscle spasm. Cardiovascularly, severe vasodilation and increased vascular permeability and third spacing will lead to intravascular volume depletion and hypotension. Rapid volume repletion may be needed. Epinephrine will increase vascular resistance, improve ventricular inotropy, and inhibit histamine release by the mast cells and basophils. Epinephrine and diphenhydramine may also help reverse bronchospasm; if ineffective, albuterol or other bronchodilators should be added. Methylprednisolone may help to treat persistent bronchospasm. The trachea should be intubated, if not already intubated, and the patient mechanically ventilated. Patients on β-adrenergic blockers may be resistant to epinephrine therapy and such patients should be administered glucagon 1 mg intravenously. If the patient responds, an infusion of glucagon of 1 to 5 mg/hour may be initiated. An H_2-blocker may be useful in inhibiting the effect of protamine on myo-

cardial and peripheral vascular tissues. In the event of catastrophic pulmonary vasoconstriction, nitric oxide may be effective,[37] but may not always be available. An inodilator such as isoproterenol or milrinone may be also useful to relieve pulmonary hypertension and reverse right ventricular failure.

If a patient presents with a history of prior adverse reaction to protamine, it should be determined whether the reaction was immunologically mediated. Levels of serum IgE, IgG, thromboxane, and C5a at the time of the reaction, if done, may be useful in determining the cause of the reaction. As stated above, a skin test and ELISA may not be useful in predicting which patient may be at risk for a future reaction to protamine. If a true immunologically mediated anaphylactic reaction occurred to protamine, then it would be best to avoid its use. When the cause of the reaction is uncertain, some people advocate pretreatment of the patient with steroids and histamine (both H_1 and H_2) blockers and slowly administering protamine. But literature basis of such regimen is lacking.

Currently, there are no clinically available alternatives to protamine. However, several modalities are under investigation. Platelet factor 4 (PF4) is a heparin-binding protein that is stored in the α granules of platelets and released during platelet aggregation.[38] Recombinant PF4 (rPF4) in a dose of about 3:1 is effective in returning the ACT to the baseline.[32,38] Unlike excess protamine dose, which can lead to platelet dysfunction and ACT prolongation, rPF4 does not have an adverse effect on platelet function or ACT up to a dose of 7:1 (rPF4:heparin) (maximum dose tested).[32] Platelet factor 4 may thus be a potential alternative to protamine with fewer side effects.

Hexadimethrine is a basic compound that can neutralize heparin and is 1.1 to 2.0 times more potent than protamine.[39] Rapid administration of hexadimethrine may cause systemic hypotension and pulmonary hypertension. In animal studies, hexadimethrine demonstrated glomerular toxicity.[40,41] Hexadimethrine has a wider therapeutic window than protamine, and ACT prolongation is not seen until the hexadimethrine:heparin ratio is 5:1 or greater.[32]

Heparinase is an enzyme that is purified from the bacterium *Flavobacterium heparinum* and cleaves the antithrombin III binding site of heparin.[42] It is currently used in in vitro assays in association with the thromboelastography and the heparinase-ACT measurement. Clinical efficacy of heparinase in reversing heparinization in cardiac surgical patients remains to be demonstrated.

Lastly, the successful use of a heparin removal device (HRD) in 2 cardiac surgical patients with known anaphylactoid reactions to protamine has been reported.[43] This device uses a venovenous circuit with plasma separation. The separated plasma passes over a poly-L-lysine-agarose surface, which binds and removes heparin, and is then recombined with the blood cells, before returning to the patient's right atrium. The veno-

venous circuit can operate at a flow rate of 1400 mL/min and returns the ACT and other coagulation parameters to near baseline values in about 30 minutes in adult patients without significant destruction of blood cells or decrease in procoagulants. Further clinical trials will be needed before the device can become available for routine use.

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