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REVIEW ARTICLE

What's fishy about protamine? Clinical use, adverse reactions, and potential alternatives

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Abstract

Protamine, a highly basic protein isolated from salmon sperm, is the only clinically available agent to reverse the anticoagulation of unfractionated heparin. Following intravenous administration, protamine binds to heparin in a nonspecific electrostatic interaction to reverse its anticoagulant effects. In clinical use, protamine is routinely administered to reverse high-dose heparin anticoagulation in cardiovascular procedures, including cardiac surgery with cardiopulmonary bypass. Despite the lack of supportive evidence regarding protamine's effectiveness to reverse low-molecular-weight heparin, it is recommended in guidelines with low-quality evidence. Different dosing strategies have been reported for reversing heparin in cardiac surgical patients based on empiric dosing, pharmacokinetics, or point-of-care measurements of heparin levels. Protamine administration is associated with a spectrum of adverse reactions that range from vasodilation to life-threatening cardiopulmonary dysfunction and shock. The life-threatening responses appear to be hypersensitivity reactions due to immunoglobulin E and/or immunoglobulin G antibodies. However, protamine and heparin-protamine complexes can activate complement inflammatory pathways and inhibit other coagulation factors. Although alternative agents for reversing heparin are not currently available for clinical use, additional research continues evaluating novel therapeutic approaches.

KEYWORDS
adverse drug reactions, anaphylaxis, anticoagulation reversal, cardiopulmonary bypass, protamines

1 | INTRODUCTION

In clinical practice, the availability of specific antagonists of anticoagulants is important for clinical management as bleeding is a significant side effect of anticoagulation. Most recently reported strategies for reversal have focused on oral anticoagulants. However, despite prior efforts to develop suitable alternatives, protamine is the only currently available agent to reverse heparin. As a basic polypeptide isolated from fish milt, protamine has been reported to produce a broad spectrum of adverse reactions, including cardiovascular effects, hypersensitivity responses, and direct effects on hemostatic function. After cardiovascular surgery, it is a mainstay of therapy to neutralize

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high-dose unfractionated heparin (UFH) levels in a patient population at high risk of bleeding and cardiopulmonary dysfunction. Although multiple protocols are reported for protamine dosing to reverse heparin following cardiac surgery, most studies suggest that dosing strategies based on the total dose of heparin and duration of cardiopulmonary bypass (CPB) may reduce bleeding. There are also conflicting reports regarding the ability of protamine to reverse low-molecular-weight heparin (LMWH). Despite prior efforts to develop alternatives to protamine for anticoagulation reversal, additional studies with several novel molecular entities for heparin reversal are under investigation. This review article will examine protamine's mechanism of action, clinical use, dosing strategies, adverse reactions, and current evaluation of potential alternatives.

2 | PROTAMINE'S STRUCTURE AND MECHANISM OF ACTION

Protamines are histones, highly basic arginine-rich polypeptides that exist in nature to provide structural integrity to DNA in cell nuclei. Protamine is a heterogeneous mixture of highly basic molecules due to the presence of up to 70% arginine and other basic amino acids [1]. The mean molecular weights of these proteins are 4000 to 5000 Da, and they comprise 50 to 110 amino acids [1]. The guanidino groups of arginine are available for binding to the acidic heparin molecule [2]. Pharmaceutical protamine is a mix of highly basic polypeptides (pKA, ~11.5) available as a sulfate or chloride for intravenous use [1]. Protamine is also combined with insulin as neutral protamine Hagedorn (NPH) and protamine-zinc insulin to retard absorption and prolong the duration of effect with parenteral administration. Commercially available protamine is purified from salmon sperm and potentially other fish gonads (milt), a product readily obtainable due to the extensive aqua farming of salmon and other fish.

Protamine sulfate is only administered by an intravenous route for anticoagulation reversal and binds electrostatically to UFH to rapidly reverse its effect. As a result, the half-life is relatively short and estimated to be approximately 5 minutes when administered intravenously [3]. Dosing strategies for protamine administration depend on the level of UFH and patient population and will be reviewed as follows.

3 | DOSING STRATEGIES FOR UNFRACTIONATED HEPARIN ANTICOAGULATION REVERSAL

3.1 | Hospitalized patients

Reversal of UFH in a hospitalized setting in nonsurgical patients is infrequently required due to the short half-life of 60 to 90 minutes [4]. In this setting, therapeutic heparin levels range from 0.3 to 0.7 units/mL [5]. Most dosing strategies in nonsurgical patients follow procedures such as pacemaker insertion, which can be performed with a protamine dose of 25 to 50 mg, often based on empiric dosing strategies for UFH of approximately 1-mg protamine per 100 units of UFH administered [6,7].

Guidelines for heparin reversal and protamine dosing are reported for patients with intracranial hemorrhage receiving UFH and recommend intravenous protamine based on the heparin dose administered over the prior 2 to 3 hours using the dosing strategy of a ratio of 1 mg for every 100 units, with a maximum single dose of 50 mg (strong recommendation, moderate-quality evidence), activated partial thromboplastin time elevation, and an additional protamine dose of 0.5 mg per 100 units of UFH (conditional recommendation, low-quality evidence) [8,9].

3.2 | Reversal of low-molecular-weight heparin

Unlike UFH, LMWH has a longer half-life, with prolongation of levels in patients with renal dysfunction [10]. However, despite the commonly believed perspective, protamine is not a complete reversal agent for the anti-Xa effect of LMWH, which led to protamine delivery to a space shuttle for an astronaut receiving LMWH for potential reversal [11]. Schroeder et al. [12] reported that protamine neutralized larger UFH fragments with a mean molecular weight of 15,000 Da but not LMWH with a mean molecular weight of 5000 Da because protamine neutralization largely depends on molecular size. LMWHs contain small oligosaccharides and with different sulfonation that cannot be effectively neutralized with protamine [12,13]. The cationic charge density of protamine is not sufficient to generate stronger electrostatic bonding with LMWHs, ultra-LMWHs, and fondaparinux to form a stable complex and neutralize their anticoagulant activity [14]. Despite the lack of supportive evidence regarding protamine's effectiveness to reverse LMWH, it is recommended in guidelines with low-quality evidence [8,9].

3.3 | Patients following cardiac surgery and extracorporeal circulation

In cardiac surgical patients, anticoagulation during CPB is maintained at higher levels using activated clotting time (ACT) monitoring. During CPB, target ACT levels are 400 to 480 seconds, which are associated with anticoagulation levels of UFH that range between 2 and 6 units/mL [15–17]. Despite the early use of ACT monitoring suggesting that heparin levels directly correlate with ACT values, the relationship is not linear and may not consistently correlate with UFH levels during or after separation from CPB [18]. The different methods of UFH reversal with protamine are described as follows.

3.4 | Empiric protamine dosing

In cardiac surgical patients, some institutions still use empiric dosing strategies for UFH reversal of 1 mg of protamine per 100 U of UFH

administered, often including doses used in priming the CPB circuit and additional doses administered during CPB [17]. However, this reversal strategy does not consider either heparin consumption or metabolism during CPB and risks the potential coagulopathy from excess protamine administration. Excess protamine's potential adverse effects include prolongation of the ACT, platelet and coagulation inhibition, and increased bleeding risk. Of note, the lowest ACT value occurs when the exact stoichiometry of heparin-protamine ratios is administered and is reported to contribute to coagulopathy when the dosing ratio of protamine-heparin is >1.3 [19].

3.5 | Modifications of empiric dosing: avoiding excess protamine

Higher total protamine doses are associated with increased bleeding and transfusions [20]. A clinical trial of 96 patients randomized to a low reversal ratio of 0.8 or a higher ratio of 1.3 for coronary revascularization with CPB reported that 24-hour postoperative bleeding was decreased in patients in the 0.8-ratio group (470 vs 615 mL; p = .021) receiving lower total doses (329 ± 95 vs 539 ± 117 mg) [21]. Although ACT values after reversal were similar, thrombin generation was higher with lower dosing (38 ± 40% vs 6 ± 9%; p = .001) and plasma and platelet transfusions were decreased (0% vs 11% and 6% vs 21%, respectively) in the lower dosing group [21].

3.6 | Pharmacokinetic protamine dosing

Pharmacokinetic dosing is based on specific protamine doses administered to match circulating heparin levels using calculations based on the pharmacokinetics of UFH and timing of administration. These dosing algorithms are predicated on calculation of heparin levels and aim to avoid excess dosing in cardiac surgical patients [22]. One report reduced the ratio of protamine administration from 0.81 to 0.49, resulting in reduced postoperative bleeding to mean levels of 480 mL compared with 694 mL in the control group [23].

3.7 | Point-of-care protamine titration

Point-of-care tests can calculate UFH levels based on protamine titration. These tests are used to maintain target UFH levels and calculate the remaining concentrations following separation from CPB to determine the optimal protamine dose. The benefit of this approach is that it avoids excess protamine administration for heparin neutralization. Several point-of-care assays in cardiac surgery are used, including Hepcon/HMS systems [17,24,25]. A limitation of the heparin-protamine titration is its insensitivity to low levels of heparin that may occur in patients who develop heparin rebound [25].

3.8 | Bleeding following CPB separation after protamine administration

Despite anticoagulation reversal following ACT normalization, patients bleed for multiple reasons due to an acquired coagulopathy [24]. Some clinicians may administer additional protamine using 50-mg to 100-mg doses when there are multiple causes of coagulopathy-induced bleeding and hemostatic function (unrelated to heparin reversal) that include platelet dysfunction, dilutional changes, low fibrinogen levels, and fibrinolysis. When repeat doses of protamine are empirically administered for bleeding, protamine accumulation may contribute to bleeding in the setting of hemostatic changes after cardiac surgery and CPB.

3.9 | Heparin rebound, detection, and management strategies

Heparin rebound is defined as increased anticoagulation due to heparin effects following complete reversal by protamine and normalization of the ACT after separation from CPB. Heparin rebound can occur with increasing levels 2 to 3 hours after administration of the initial dose of protamine due to release of heparin from multiple sites, including those bound to tissues or proteins, heparin-protamine complexes, and reinfusion of unwashed blood from the extracorporeal circuit [17,26]. Although cardiac surgical centers routinely use normalization of ACT after heparin neutralization as evidence of complete reversal, the ACT is insensitive to low heparin concentrations that can occur during rebound [17]. Furthermore, plasma levels reported for heparin rebound range from 0.05 to 0.1 U/mL, levels that are not measurable using most point-of-care assays. Therefore, according to point-of-care assays, heparin rebound following CPB will potentially occur despite "effective" UFH reversal [17,22,25,26].

One of the suggested management strategies is to use a 25-mg/h protamine infusion for 4 to 6 hours after administration of the initial protamine reversal dose to prevent heparin rebound. In a cardiac surgical study, Teoh et al. [26] evaluated 300 patients randomized to receive an infusion of 25 mg/h of protamine or no protamine for 6 hours or placebo after surgery. The authors evaluated anti–factor Xa activity and thrombin times and reported that all patients developed heparin rebound from 1 to 6 hours after surgery, except for those in the protamine infusion group. There was a 13% reduction in chest tube drainage after surgery but no difference in transfusions, and no adverse events were attributable to the additional protamine infusion [26].

4 | ADVERSE REACTIONS TO PROTAMINE

Multiple life-threatening adverse drug reactions have been reported following the administration of protamine, including hypotension, pulmonary hypertension, bronchospasm, systemic hypotension, and/or shock [27,28]. Many reports have described potential mechanisms

of protamine reactions. Efforts have attempted to classify protamine reactions based on clinical presentations and adverse hemodynamic effects, including vasodilation, acute pulmonary vasoconstriction, right ventricular failure, and/or shock [27,29,30]. However, the critical question in defining adverse reactions to protamine is whether the reaction is due to hypersensitivity and immunologic responses or direct effects such as myocardial depression or vasodilation.

Adverse reactions can also occur to any intravenously administered agent, including hypersensitivity due to immunologic reactions to protamine and heparin. The more common adverse immunologic reaction to heparin is heparin-induced thrombocytopenia, a pathophysiologic response due to the formation of an immunospecific immunoglobulin G (IgG) antibody directed against a platelet factor 4 (PF4) that is released from stored alpha granules of platelets [31]. Protamine can produce multiple adverse reactions following intravenous administration, which include nonimmunologic mast cell degranulation, activation of complement due to protamine-UFH binding, and anaphylaxis [27]. Protamine can also bind to endothelial cell surfaces that contain negatively charged glycosaminoglycans, including heparan sulfate and chondroitin sulfate [32].

Due to the multiple reports regarding adverse reactions to protamine, the published literature is perplexing regarding the underlying mechanism for adverse hemodynamic effects. Protamine reactions are often reported on the basis of clinical presentations, such as vasodilation, catastrophic pulmonary vasoconstriction, and shock following cardiac surgery [27]. An early classification of protamine reactions from 1985, which is still referred to, defined them as rapid administration hypotension, anaphylactoid including noncardiac pulmonary edema, and catastrophic pulmonary vasoconstriction [30]. However, 38 years later, multiple studies have better defined the complex adverse reactions associated with direct and indirect effects of protamine, which will be described subsequently.

In cardiac surgical patients, the temporal sequence of protamine administration follows separation from extracorporeal circulation (CPB), a vulnerable time for myocardial depression, reperfusion injury, or problems with surgical repair to occur, confounding the cause of the shock and/or myocardial dysfunction. In this setting, allogeneic blood products may be administered concomitantly or within a short time after protamine is infused. As a result, protamine may be named as the culprit antigen when multiple agents are involved, especially for anaphylaxis and hypersensitivity reactions [33]. Noncardiac pulmonary edema is also consistent with transfusion-related acute lung injury, previously due to fresh frozen plasma or other blood products, which are often administered concomitantly for bleeding management with protamine administration [34]. Due to the multiple underlying mechanisms and different etiologies regarding its adverse effects, different pathophysiologic responses implicated in protamine reactions were considered as follows.

4.1 | Protamine and hemodynamic effects

Protamine's direct effects on the heart following intravenous administration in patients vary extensively. In clinical practice, high doses of protamine are infused after weaning from CPB and potentially following aortic decannulation, although some clinicians administer an initial dose of protamine while the aortic cannula is present in case of anaphylaxis. However, the direct effects of protamine on hemodynamic changes in patients vary and are frequently reported in patients following cardiac surgery when there are multiple potential causes of hypotension and cardiovascular dysfunction. Although, as a potential allergen, protamine can cause acute cardiopulmonary dysfunction and shock, as an immediate hypersensitivity reaction, anaphylaxis, due to immunoglobulin E (IgE) and/or IgG antibodies that will be considered in more detail as follows.

4.2 | Infusion rate of protamine

Despite prescribing information that states, "Protamine sulfate injection should be given by very slow intravenous injection over a 10-minute period in doses not to exceed 50 mg" (https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=94d4f57f-940e-c0a8-e053-2a95a90ae3af&type=display), this is not consistent with clinical practices and, in particular, would pose major problems for reversing heparin after cardiac surgery and CPB where mean protamine doses range from 200 to 300 mg. Although the administration rate of protamine infusions is often suggested as a potential reason for acute reactions, this does not consistently occur even with a relatively rapid administration. From prior surveys, over 60% of clinical practices for managing cardiac surgical patients infuse total initial protamine dosing over 5 to 10 minutes [17].

4.3 | Direct histamine release by protamine

Protamine can directly activate human connective tissue mast cells to release histamine, although this occurs at concentrations not achieved with standard-dose administration in clinical settings [35]. Other studies examining the *in vitro* effects of protamine or protamine-heparin complexes also do not release histamine from human lung mucosal mast cells or basophils [36,37], further suggesting that this is an unlikely mechanism.

5 | ANAPHYLAXIS AND HYPERSENSITIVITY REACTIONS TO PROTAMINE

Multiple mechanisms are implicated in the pathophysiology of protamine reactions in patients, including nonimmunologic mast cell degranulation/activation, complement activation from IgG or direct activation due to heparin-protamine complexes, and IgE and/or IgG antibody formation. Many reported studies are in animal models, with effects that may not occur in human hypersensitivity reactions. As a result, this focus will be on *in vitro* studies with human tissues and responses.

5.1 | Direct effects of protamine on mast cells and basophils

Although highly basic molecules can cause direct, nonimmunologic release of histamine from connective tissue mast cells *in vitro*, studies on human lung mast cells (mucosal mast cells) do not demonstrate histamine release or mast cell degranulation to either protamine or protamine-heparin complexes [36]. Although prior studies report that protamine can degranulate human connective tissue (skin) mast cells *in vitro*, this occurs at concentrations not achieved clinically [35]. Furthermore, protamine is administered intravenously in only heparinized patients and not without systemic heparin levels.

5.2 | Anaphylactic reactions to protamine

Anaphylaxis is an acute hypersensitivity reaction, with multiple definitions in the literature. Based on a National Heart Lung Blood Institute consensus, the clinical criteria for diagnosing anaphylaxis is "a serious allergic reaction that is rapid in onset and may cause death," as previously described in a symposium summary report [38]. The immunologic release of multiple mediators produces acute cardiopulmonary dysfunction. IgE-mediated anaphylaxis is a common cause of anaphylaxis due to mast cell and basophil release. However, IgE as the cause of anaphylaxis is only characterized in 60% of anaphylactic reactions. Other important pathophysiologic mechanisms include IgG-mediated reactions and potential direct or indirect complement activation discussed as follows.

The human response to anaphylaxis is characterized by distributive shock, which is characterized by vasodilation, also described as vasoplegia or vasogenic shock, that occurs following protamine exposure and may have a rapid onset within 5 minutes for IgE-mediated reactions but could take up to 20 minutes [27]. Most cardiopulmonary effects of anaphylaxis to protamine administration are reported in cardiac surgical patients with extensive indwelling hemodynamic monitors, including arterial catheters, pulmonary artery catheters, transesophageal echocardiography, and endotracheal intubation with mechanical ventilation, airway pressure, and volume monitoring. Perioperative anaphylaxis includes a spectrum of clinical manifestations ranging from decreased blood pressure to vasodilatory shock, bronchospasm, or multiple arrhythmias [39,40]. Echocardiographic findings in cardiac surgical patients developing anaphylaxis are hyperdynamic ventricular function and hypovolemia in classic IgE-mediated anaphylaxis [27].

5.3 | Antibody formation to protamine

IgE formation has been documented in multiple studies of protamine as an important cause of anaphylaxis [41–44]. However, IgG is another important mechanism proposed to explain anaphylaxis without the ability to determine an IgE-mediated mechanism [45]. Multiple patients sensitized to protamine also form specific IgG antibodies, as previously reported in an evaluation of patients following protamine anaphylaxis [41]. IgG-mediated anaphylaxis, also previously called aggregate anaphylaxis, can occur due to volume expanders (dextrans) or proteins and has been reported to explain hypersensitivity reactions [27]. Additional reports of perioperative anaphylaxis include the role of antigen-specific IgG antibodies also been reported in patients. Jönsson et al. [46] evaluated 86 patients with anaphylaxis and reported that IgG, platelet-activating factor, and neutrophil activation correlated with anaphylaxis severity, consistent with IgG-mediated anaphylaxis. The term anaphylaxis was first coined by Richet to define anaphylaxis following an IgG-mediated response to sea anemone toxin [47].

Protamine-specific IgG antibodies are also reported as the mechanism for complement activation and protamine reactions [27]. In patients with diabetes receiving protamine-insulin injections, antiprotamine IgE antibody levels were a risk factor for acute protamine reactions, as were antiprotamine IgG antibodies [41]. Based on these reported studies, complement activation by IgG-specific antiprotamine antibodies is likely a mechanism for the catastrophic pulmonary vasoconstrictive reactions.

Antibodies to heparin-protamine complexes can activate platelets, as noted from a prospective heparin-induced thrombocytopenia study following CPB (n = 500 patients) [48]. High-titer antibodies (1:14 744 mean) developed to heparin-protamine complexes that demonstrated heparin-dependent binding and platelet activation in response to protamine. Although the antibodies did not cross-react to PF4/heparin complexes, they did cross-react to protamine-containing insulins [48]. In another report of 591 patients, 9.6% (n = 57) of patients developed antibodies to heparin-protamine complexes after cardiac surgery and 26.6% (n = 154) of them developed antibodies by day 10 [49]. Diabetes was a risk factor for antibody formation and represented a postoperative risk factor for thrombosis [49,50].

5.4 | Pulmonary vasoconstriction

A reported life-threatening response to protamine administration after CPB is acute pulmonary vasoconstriction and subsequent right ventricular failure. This life-threatening reaction is called catastrophic pulmonary vasoconstriction [29]. The right ventricle normally ejects against a low resistance system as mean pulmonary artery pressures in healthy subjects at rest are ≤20 mm Hg [51]. When acute pulmonary vasoconstriction occurs, the mean pulmonary artery pressures increase several-fold, fluid backs up in the right ventricle causing acute right ventricular dilatation; the intraventricular septum shifts toward the left ventricle, reducing ventricular filling; and the patient experiences acute cardiogenic shock (Figure). This hypersensitivity reaction appears to be due to complement activation, anaphylatoxin generation (C5a), platelet activation, and neutrophil-mediated release of the potent pulmonary vasoconstrictor thromboxane [52]. Although protamine-heparin complexes can also directly activate complement, this appears to occur in animal models. If direct activation of complement by protamine-heparin complexes is a mechanism for catastrophic pulmonary vasoconstriction, then as a direct affect, the reactions should be more common. However,

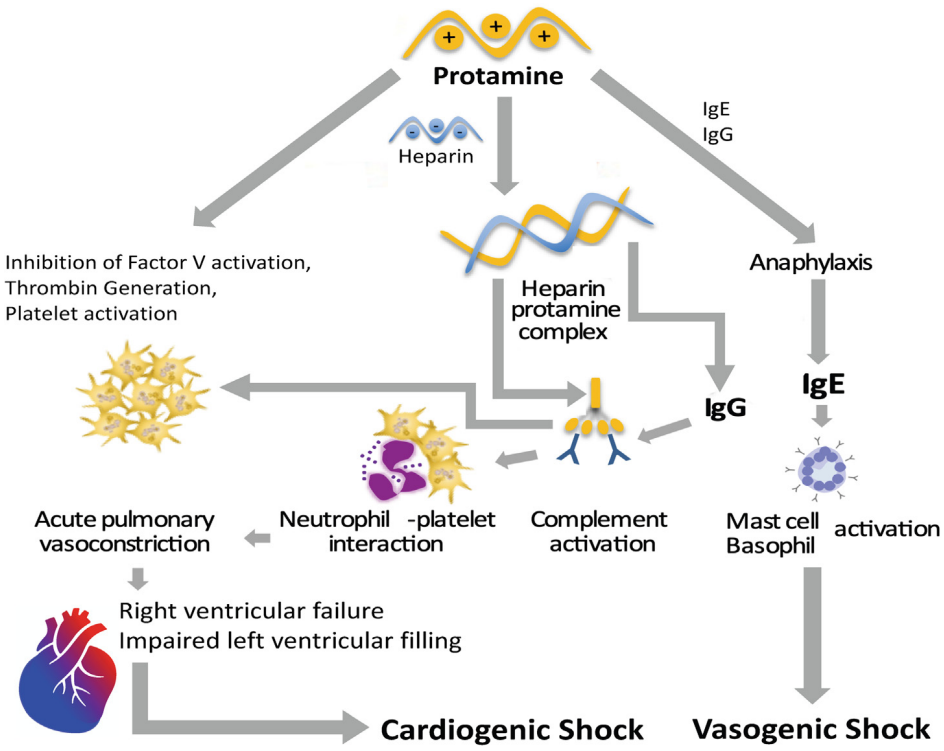

FIGURE Protamine, a basic polypeptide, binds to unfractionated heparin, in an electrostatic interaction (polyionic-polycationic), to reverse its anticoagulant effects. In sensitized patients, protamine may also be a cause of an antigen-triggering anaphylaxis due to immunoglobulin E (IgE) and/or immunoglobulin G (IgG) antibodies. The IgE responses trigger mast cell and basophil activation, release of multiple mediators, and produce anaphylaxis and vasogenic (distributive or vasodilatory) shock. IgG antibodies also bind to complement, releasing complement anaphylatoxins that activate both neutrophils and platelets and generate thromboxane, which can produce acute pulmonary vasoconstriction, right ventricular failure, and ensuing cardiogenic shock. Protamine also has direct effects on hemostasis, including inhibition of factor V, thrombin generation, and/or platelet activation.

there may be other explanations as to why these events are rare even if this mechanism is involved, particularly patient-related factors that may predispose to susceptibility. Clinically, these life-threatening pulmonary hypertensive responses are rare and unpredictable and are consistent with anaphylaxis [52].

5.5 | Direct complement activation by protamine

Following the administration of protamine, heparin binds to it as an acid-base (electrostatic) interaction. The result is the formation of a polyanionic-polycationic complex inactivating UFH that also activates complement by C1 esterase binding. This sequence initiates the classical complement pathway to form C5a and C3a anaphylatoxins that activate both neutrophils and platelets [53]. In addition, Morel et al. [52] reported pulmonary vascular sequestration of neutrophils following protamine injection in animal models. Although these effects may be responsible for potential hypotensive reactions following protamine infusions, this effect is inconsistent in patients.

5.6 | Direct effects of protamine on coagulation and clot structure

Protamine exhibits intrinsic anticoagulant properties by direct effects but also potentially due to excess protamine administration beyond the stoichiometric doses needed for UFH neutralization [54,55]. Protamine can increase the ACT, the test used to determine reversal, and is reported to increase bleeding in cardiothoracic surgery [19,56]. High-dose protamine also inhibits factor V activation by inhibiting thrombin generation, FXa, and tissue factor formation in human plasma [54,57,58]. High protamine concentrations also generate thicker fibrin fibers that alter clot morphology, increase fibrinolytic susceptibility, and alter clot mechanical properties following UFH neutralization [59,60]. Protamine is also a protease inhibitor that inhibits carboxypeptidase N, an enzyme that removes a C-terminal arginine molecule from complement anaphylatoxins C3a/C5a and bradykinin to inactivate their biologic effects [61].

5.7 | Patients at risk for protamine reactions

5.7.1 | Patients taking protamine-containing insulins

Patients at risk of protamine reactions include those previously sensitized due to receiving protamine-containing insulins in which protamine is added to retard insulin absorption, mostly including NPH insulin. An initial report noted that 4 of 15 patients with diabetes on NPH insulin developed anaphylaxis after protamine administration following cardiac catheterization [62]. Larger subsequent publications on cardiac surgery reported an incidence of 0.6% (1 of 160) to 2% (1 of 50) of protamine reactions in NPH insulin–dependent diabetic patients, a rate 10 to 30 times greater than that among non–NPH insulin–dependent patients from 4796 cardiac surgical patients evaluated, with an incidence of 1 in

1500 [37,44]. Although NPH insulin was previously the most commonly used intermediate- to long-acting insulin until the introduction of the longer-acting insulin analogs (insulin glargine in 2000 and insulin detemir in 2005), NPH insulin is far less used currently. As a result, there is likely far less exposure and potential sensitization and potentially a decrease in risk for prior sensitization.

5.7.2 | Vasectomized patients

Another group of patients allegedly at increased risk of reactions to protamine are vasectomized patients as they develop autoantibodies to their sperm. Because the sperm and testes are isolated from immunologic recognition, following vasectomy and vas deferens interruption, blood is exposed to these otherwise immunologically isolated tissues. As a result, vasectomized patients develop antisperm antibodies that can cross-react with protamine [63]. Antisperm antibodies have the potential to also cross-react with protamine as a potential cause of hypersensitivity reactions [64]. However, in a prospective report of 16 cardiac surgical patients who had prior vasectomies, none of the patients developed adverse reactions after protamine administration for heparin reversal, suggesting a poorly defined prevalence for this cross-reaction in patients [44].

5.7.3 | Fish-allergic patients

Patients with a history of allergy to fish have also been suggested to be at potential risks of protamine reactions. However, protamine is purified from salmon's milt (testes), and patients who eat fish do not routinely consume milt. Alternately, protamine is purified from fish that may be contaminated with residual fish proteins. Evidence supporting the increased risk of protamine reactions in fish-allergic patients is lacking and is limited to case reports [27]. A prospective study of 6 fish-allergic patients noted no reaction to protamine [44]. Patients with a history of multiple drug allergies or food hypersensitivity may also be allergic to other food allergens, which indicate their allergy history rather than a protamine cross-reactivity.

5.7.4 | Screening patients for protamine allergy

Screening patients receiving protamine for potential antiprotamine antibodies is not currently performed as there are no clinically available tests, the risk is relatively low, and no potential alternative heparin reversal agents are available. Further, as previously noted, both IgE and IgG antibodies appear responsible for many clinical presentations of hypersensitivity to protamine. Protamine used clinically is also a heterogeneous mix of different macromolecules obtained from the protein purification process, and whether the clinically used preparation of protamine is an incomplete or univalent antigen that must first combine with heparin or another potential macromolecule to become a complete, multivalent antigen is not known.

5.7.5 | Managing the patient following a protamine reaction

For patients who manifest allergic responses to protamine characterized by urticaria or mild blood pressure changes, protamine infusion should be stopped, and standard symptomatic management should be followed for any allergic reaction. However, patients developing acute cardiopulmonary dysfunction and shock, classic manifestations of anaphylaxis, epinephrine, and standard resuscitative measures should be followed based on managing anaphylaxis and/or cardiopulmonary life support [33]. Protamine is more commonly administered in an operating room or intensive care unit setting where patients have indwelling vascular access for rapid resuscitation and standard hemodynamic monitoring. Cardiac surgical patients are more intensively monitored with intra-arterial catheters, central vascular access, and transesophageal echocardiography to allow rapid resuscitation. In this setting, backup mechanical cardiovascular support, including venoarterial extracorporeal membrane oxygenation or reinstitution of CPB, can be implemented for refractory shock [27,33].

6 | ALTERNATIVES TO PROTAMINE

Due to the adverse reactions that protamine can cause, efforts to develop a clinical alternative have continued. The ideal agent should be able to reverse the activity of LMWHs and other heparin-based anticoagulants in the case of a bleeding episode. Although alternative agents have been previously used and/or studied in clinical trials, none are available or approved for clinical use [65–67]. Prior attempts to develop protamine replacement therapeutics have had limited success due to limited clinical need for an alternative, costs, and extensive clinical experience using protamine as the only agent available for many decades for heparin reversal.

6.1 | Hexadimethrine

Hexadimethrine, like protamine, is a polycation but has a synthetic origin. It is effective for reversing heparin and has been previously administered in patients allergic to protamine [68]. However, it can cause similar side effects to those of protamine, which include systemic hypotension when administered rapidly and pulmonary hypertension in certain patients. Unfortunately, hexadimethrine is no longer available for use.

6.2 | PF4

PF4 is stored in platelets and is released during platelet activation. PF4 binds to heparin to neutralize its effects and is a potential cause of heparin resistance with thrombocytosis. PF4 produced by recombinant DNA techniques has been studied following cardiac catheterization and cardiac surgery in patients and has been shown to be effective [65,69]. However, it was never approved for clinical use.

6.3 | Heparinase

Heparinase cleaves alpha glycosidic heparin linkages into fragments without anticoagulant activity [70]. Although studied in patients following cardiac surgery, bleeding was greater in the heparinase-treated patients, and it was never approved for patient use [66].

6.4 | Universal heparin reversal agent

Universal heparin reversal agent (UHRA) is a synthetic polycation that can neutralize all types of heparins, including LMWH [60]. Unlike protamine, which has direct anticoagulant effects that can potentially interfere with fibrin polymerization, UHRA does not affect fibrin clot morphology. UHRA has been studied in animal models and in human blood [60]. UHRA reverses the anticoagulation activity of UFH and LMWH without associated intrinsic anticoagulant activity and was well tolerated in animal studies [60]. Further drug modifications are underway, and no clinical studies have yet been performed.

6.5 | Andexanet-alfa

Andexanet-alfa can reverse LMWH and UFH [71]. In the updated Andexanet Alfa, a Novel Antidote to the Anticoagulation Effects of Factor Xa Inhibitors-4 (ANNEXA-4) trials, 20 patients in the safety population and 16 patients in the efficacy population receiving enoxaparin were studied for reversal. Others have also reported its ability to reverse LMWH [72]. However, because of the small number of patients evaluated in the study, andexanet-alfa only received Food and Drug Administration approval for apixaban and rivaroxaban reversal. As noted, andexanet-alfa reverses UFH and is a potential cause of heparin resistance when used for patients requiring cardiac surgery [73,74].

6.6 | Other heparin reversal agents

Additional heparin reversal agents have been tested preclinically as well as in human blood as an alternative to protamine [60,75]. These include protamine variants (+18BE and +18RGD) [76,77] with lesser side effects than protamine and synthetic peptides such as PM102/HepArrest [78,79], Dex40-GTMAC-3 [80], and heparin-binding copolymer. Other recent investigations include the development of porous cationic polymers, which can selectively sequester heparins demonstrating reversal activity in animal studies [81].

7 | CONCLUSION

Protamine is the only clinically available agent for UFH reversal. Although administered for LMWH reversal, it is not a complete reversal agent. In cardiovascular surgical patients, protamine is routinely used to reverse UFH following procedural intervention and termination of CPB. Different dosing strategies have been reported in the cardiac surgical setting based on empiric dosing, prior algorithms, and laboratory measurements. Multiple adverse hypersensitivity drug reactions that contribute to acute cardiopulmonary dysfunction and shock following its administration have been reported. The life-threatening reactions appear to be hypersensitivity reactions. As reviewed, protamine reactions' underlying pathophysiologic mechanisms include nonimmunologic and immunologic responses. However, the unpredictable onset of acute cardiopulmonary dysfunction and shock suggests that hypersensitivity reactions are the main causes of acute hemodynamic compromise. Despite attempts to develop an alternative agent for UFH reversal, currently, no approved drug is available, although novel additional studies evaluating therapeutics are underway.

AUTHOR CONTRIBUTIONS

All authors met authorship criteria and participated significantly in the report. J.H.L. wrote the first draft, and all authors reviewed and revised the manuscript. All authors read and approved the final manuscript and revision.

DECLARATION OF COMPETING INTERESTS

J.H.L. serves on the Steering Committees for Merck, Octapharma, and Werfen. K.G. receives grant funding through his institution from Octapharma AG. J.N.K. is an inventor of patents related to heparin neutralization filed by the University of British Columbia and is a Tier 1 Canada Research Chair in Immunomodulating Materials and Immunotherapy. T.I. has participated in advisory boards of the Japan Blood Products Organization, Toray Medical, and Asahi Kasei Pharmaceuticals.

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