Neutralization of Heparin by Protamine
Time for a Change?
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The DNA of eukaryotic chromosomes forms a complex called chromatin with histones and other nucleic acid–binding proteins. In certain structures like sperm heads in which chromatin is present in a very condensed form, histones are replaced by smaller proteins, the protamines. The structure–function relations of protamines have been studied in detail: they contain between 31 and 34 amino acid residues, two thirds of them basic, most of them arginine. When complexed to nucleic acids, protamines are folded into four α-helical domains, the cross-linking of DNA double helices being achieved through arginine-phosphate interactions.1,2

A therapeutic role for protamine was first envisioned by Hagedorn et al3 who observed that protamine-insulin suspensions are relatively insoluble in water or serum at neutral pH. This contrasts with regular insulin, which because of its acidic isoelectric point is extremely soluble under these conditions. The relative insolubility of protamine-insulin slows the rate at which the hormone is absorbed; consequently, its biological activity persists for 24–36 hours after subcutaneous injection (for comparison, the half-life of regular insulin after subcutaneous injection is about 4 hours).3,4 Because insulin and heparin have similar electric charges, Chargaff and Olson5 speculated that protamine might also prolong the anticoagulant effect of heparin. This, however, is not the case, because protamine efficiently inhibits the action of heparin both in vitro and in vivo.5,6 Subsequently, neutralization of the anticoagulant activity of heparin with protamine became standard practice after extracorporeal circulation for open-heart surgery.7

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Protamine toxicity has received much attention (for a review, see Reference 8). The early observation that its rapid intravenous administration can cause arterial hypotension, hyperpnea, and thrombocytopenia in the dog8 was followed by studies of cardiovascular protamine toxicity after extracorporeal circulation in humans.10,11 Jastrzebski et al10 observed a 37% increase in the mean pulmonary artery pressure of 15 patients who received protamine (6 mg/kg over a period of about 5 minutes) immediately after bypass. More recently, Lowenstein et al11 described five patients who experienced severe systemic hypotension and pulmonary vasoconstriction with 20–30 mg intravenous bolus doses of this compound. Subsequent studies involving 904 consecutive patients revealed that the incidence of protamine-induced pulmonary artery vasoconstriction after bypass was about 1%,12 and that this reaction was accompanied by the generation of thromboxane and C5a, the anaphylatoxin derived from the fifth component of complement.13 In non-diabetic patients, the pathogenesis of protamine-induced pulmonary artery vasoconstriction is still poorly understood. In diabetic patients who have IgE or IgG antibodies against protamine because they have been treated with protamine-insulin preparations, the syndrome of pulmonary artery vasoconstriction and systemic hypotension is probably mediated by antibody-dependent reactions.14

This issue of Circulation contains two articles by Montalescot et al15,16 both addressing the problem of the lung response to heparin-protamine administration in the instrumented sheep model. Studies using this model have certainly yielded insight into endotoxin-induced lung dysfunction in patients with sepsis.17 However, in the case of pulmonary hypertension caused by heparin-protamine administration, conclusions obtained from sheep experiments might not be applicable to the reaction seen in humans. In contrast to sheep, in which the incidence of acute pulmonary hypertension accompanying reversal of the effects of heparin by protamine approaches 100%, only 1% of the patients receiving protamine after cardiopulmonary bypass develop significant pulmonary hypertension.12 Of these, only a third require reinstitution of extracorporeal circulation.12 Why is it that pulmonary reactions to heparin-protamine administration have such a different incidence in humans and sheep? It seems likely that much of the thromboxane (and presumably the pulmonary hypertension) generated after heparin-protamine interactions derives from pulmonary intravascular macro-
phages (PIMs), which are perivascular cells found in large numbers in the pulmonary microvasculature of certain animal species (e.g., sheep, cows, and pigs) but not of humans. Chang and Voelkel have commented on the marked similarities in pulmonary pressor response and mediator release in sheep receiving heparin-protamine and those receiving circulating microparticles designed to activate PIMs.

The results presented in the first article by Montalescot et al further support the conclusion that thromboxane A\textsubscript{2}/endothelin receptor activation is an important pathogenic mechanism for the pulmonary hypertension and arterial hypoxemia observed following protamine reversal of heparin anticoagulation in the instrumented sheep. As opposed to control animals, sheep pretreated with SQ 30,741 (a thromboxane A\textsubscript{2}/endothelin receptor antagonist) had minimal pulmonary pressor response and no hypoxemia. Whether pretreatment with the antagonist influences indicators of lung injury (e.g., lymph protein clearance, wet–dry weights, etc.) has not been examined. Because SQ 30,741 has a short half-life, the authors suggest that it could be used clinically just before protamine administration without causing potentially morbid postoperative platelet dysfunction. This is not a trivial concern, as postoperative hemorrhage after cardiac surgery is certainly more common than catastrophic reactions to heparin-protamine.

In their second article, Montalescot et al compared the merits of different heparins (unfractionated heparin versus enoxaparin) and polycations (protamine versus polybrene) in a sheep model. Enoxaparin is a low molecular weight heparin derivative prepared by depolymerization of porcine intestinal mucosa heparin. Polybrene is the commercial name for hexadimethrin bromide, a polymer of N\textsubscript{2}N\textsubscript{2}N\textsuperscript{2}'N\textsuperscript{2}'-tetramethylhexamethylenediamine and trimethylene bromide. Whereas anticoagulation with unfractionated heparin was reversed by protamine or by polybrene (or when enoxaparin was reversed by protamine), reversal of enoxaparin by polybrene was not accompanied by pulmonary artery vasoconstriction or thromboxane release. No information is provided about effects of polybrene given without heparin. Toyofuku and coworkers, also working with a conscious sheep model, documented marked increases in pulmonary arterial pressure, lung lymph flow, and lymph/plasma protein ratio after infusion of 0.1 mg/kg polybrene. What are the clinical implications of these findings? Is it possible that enoxaparin (or another low molecular weight heparin) will replace unfractionated heparin as the anticoagulant in cardiopulmonary bypass. It is, however, unlikely that polybrene will again be employed for heparin neutralization. The experiments with polybrene alone suggest that small errors in calculation of the appropriate dose of polybrene could result in significant polycation-induced lung injury. In addition, polybrene nephrotoxicity is probably an insurmountable obstacle to its clinical use. During the 1950s, this polycation had its time of popularity; in certain medical centers, it almost replaced protamine as a heparin antagonist. However, several patients who received this compound developed renal failure, and as a result, polybrene manufacture for clinical purposes was discontinued in 1962. The notion that polybrene is nephrotoxic was also supported by studies in dogs, which showed that animals treated with this polycation had renal function abnormalities and histological lesions (tubular necrosis) akin to those observed in humans. Therefore, protamine (despite its undesirable effects on the pulmonary circulation) is certainly much safer than polybrene and, for the time being, will remain the agent of choice for neutralizing heparin after extracorporeal circulation.

References
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