Anesthesiology
1996; 85:339–46

Heparinase I (Neutralase) Reversal of Systemic Anticoagulation

Luis G. Michelsen, M.D., Mutsuhito Kikura, M.D.,† Jerrold H. Levy, M.D.,‡ Mi Kyoung Lee, M.D., Ph.D.,† King C. Lee, Ph.D.,§ Joseph J. Zimmermann, Ph.D.,§ Fania Szlam, M.S.‖*

Background: **Protamine causes multiple adverse reactions. Heparinase I, a specific enzyme that inactivates heparin, is a possible alternative to protamine. In this study, the authors examined the efficacy of heparinase I to reverse heparin-induced anticoagulation *in vitro* and compared heparinase I to protamine as an antagonist of heparin-induced anticoagulation in dogs.**

Methods: **In the *in vitro* study, blood was obtained from the extracorporeal circuits of 12 patients, and activated clotting times were determined after adding different concentrations of heparinase I. In the *in vivo* study, 24 anesthetized dogs received 300 units/kg heparin injected intravenously for 5 s, then 10 min later, 3.9 mg/kg protamine, 5–41 μg/kg heparinase I, or the vehicle (n = 4/group) were administered intravenously, and activated clotting times and hemodynamics were measured.**

Results: **In the *in vitro* study, heparin concentrations of 3.3 ± 1.0 (mean ± SD) units/ml (~0.033 mg/ml; n = 12) were reversed in the blood of patients by heparinase I at concentrations >0.490 μg/ml. In the canine study, heparinase at all doses studied and protamine effectively reversed the anticoagulating effects of heparin within 10 min of administration. Protamine produced adverse hemodynamic effects, whereas heparinase or its vehicle produced no significant change in arterial pressure.**

Conclusion: **Both heparinase I and protamine effectively reversed heparin anticoagulation. However, as opposed to protamine, heparinase I did not produce any significant hemodynamic changes when given as a bolus to dogs. (Key words: Blood, anticoagulation: heparin; heparin neutralization. Pharmacology: heparinase; protamine.)**

PROTAMINE is the only drug currently available for reversal of heparin-induced anticoagulation. The spectrum of adverse drug reactions to protamine ranges from transient hypotension (common) associated with the rate of administration to life-threatening anaphylactic reactions, which occur in 0.6–2.0% of patients receiving neutral protamine Hagedorn insulin.[1,2] Currently, there are tests to evaluate patients with immunospecific antibodies to protamine. However, even if these patients could be identified preoperatively, an adverse reaction may still occur, and there are no effective alternatives for reversing heparin-induced anticoagulation. Alternatives being investigated for the reversal of heparin-induced anticoagulation include heparin removal devices,[3,4] and platelet factor 4.[5] The current study investigates heparinase I (Neutralase, Ibex Technologies, Montreal, Quebec), a specific enzyme that inactivates heparin and that is currently under clinical development, as a possible alternative to protamine. Previous studies have found that the minimum effective dose to reverse the effects of heparin (100 and 300 units/kg) in rabbits is 5.1 and 10 μg/kg, respectively.# The objective of the first phase of this study was to determine *in vitro* the concentration-response relationship between heparinase I (Neutralase) and neutralization of heparin-induced anticoagulation in residual blood obtained from the extracorporeal circuit immediately after surgery using cardiopulmonary bypass (CPB) in humans. The objective of the second phase of this study was to determine and compare the efficacy and hemodynamic effects of heparinase I *versus*

* Assistant Professor of Anesthesiology.

† Research Fellow, Department of Anesthesiology.

‡ Professor of Anesthesiology.

§ IBEX Technologies.

‖ Research Associate.

Received from the Department of Anesthesiology, Emory University School of Medicine, Division of Cardiothoracic Anesthesiology and Critical Care, Atlanta, Georgia, and IBEX Technologies, Inc., Montreal, Canada. Submitted for publication January 23, 1995. Accepted for publication March 11, 1996. This work was done at Emory University, and was supported by a grant from IBEX Technologies, Inc. Presented in part at the annual meeting of the Society of Cardiovascular Anesthesiologists, Montreal, Canada, April 1994.

Address reprint requests to Dr. Levy: Department of Anesthesiology, 1364 Clifton Road, Atlanta, Georgia 30322.

Lee K, Zimmermann J, Lambert C, Bier C: Minimum effective dose and pharmacokinetics of heparinase I (a novel and improved heparin-reversal agent) in rabbits. Proceedings of the 16th Annual Meeting of the Society of Cardiovascular Anesthesiologists 1994; 302.

protamine during reversal of heparin-induced anticoagulation in anesthetized dogs.

Materials and Methods

Ex Vivo *Study*

Patients' Blood. Residual blood (4- or 5-ml/sample) was obtained from the extracorporeal circuits of 12 patients at the end of CPB before the administration of protamine. The patients had received heparin (from bovine lung) 400 units/kg before being placed on CPB and the CPB circuit was primed with an additional 8,000 units of heparin. The duration of CPB and the time since administration of the heparin dose varied among patients. To account for the variation in the amount of heparin present in the sample, the heparin concentration was determined as outlined later. All procedures in handling patients' blood had previously been approved by the Human Investigations Committee.

Study Design. Immediately after obtaining the blood sample, heparin concentrations were determined (Hepcon H.M.S., Hemotec, Englewood, CO). After this, heparinase I was mixed with 0.37 ml of the patients' blood in the appropriate amounts (1–2 ml) to obtain total heparinase concentrations of 0.04–0.24 IU/ml. These solutions were pipetted into cartridges and the activated clotting time (ACT) was then determined (Medtronic-Hemotec, Englewood, CO) after incubation of the blood sample at 37°C for 0, 5, 10, 15 or 20 min. The reproducibility of the ACT assay was ±12% for clotting times up to 600 s.

Study in Dogs

Animals and Surgery. Twenty-four mongrel dogs weighing 15–25 kg were used. All procedures were conducted in accordance with state and federal legislation, and approved by the Institutional Animal Care and Use Committee of Emory University.

Dogs were intravenously injected with 5 mg/kg thiopental to induce anesthesia, and general anesthesia was maintained using halothane. The dog's lungs were mechanically ventilated (Harvard respirator; endotracheal intubation) with 100% oxygen. A femoral artery was cannulated for measuring mean arterial pressure (MAP) heart rate (derived from arterial pulse), and for sampling blood. The arterial *p*H and P_{CO_2} were measured at least hourly, and respiration was adjusted to maintain *p*H and P_{CO_2} at physiologic levels. An 8F introducer was placed percutaneously in the right external jugular vein. Lactated Ringer's solution was infused *via* the side port of the introducer, as needed, to maintain central venous pressure greater than 4 mmHg. A 7F thermodilution pulmonary artery catheter (Model 93A-075, Baxter Healthcare, Santa Ana, CA) was placed through an introducer into the pulmonary artery for measurement of mean pulmonary artery pressure, pulmonary artery occlusion pressure, cardiac output and core blood temperature. Cardiac index (CI) was derived by dividing cardiac output with body surface area (determined by using the formula $0.12 \times [\text{body weight}]^{2/3}$). Indexes of systemic vascular resistance and pulmonary vascular resistance were derived according to the formulas (MAP-CVP) × 80/CI and (mean pulmonary artery pressure-pulmonary artery occlusion pressure) × 80/CI, respectively. Blood pressures were measured using pressure transducers (Model DTX disposable transducers, Viggo-Spectramed, Oxnard, CA). All hemodynamic tracings were displayed on a CRT monitor (Model 7700, Hewlett Packard, Waltham, MA) and continuously recorded on an 8-channel polygraph (Model 7758 A and B, Hewlett Packard). During the study, the core body temperature of the dogs was maintained at 36.5–38.5°C using a heating mattress (Model COM-1 RS, Am. Edwards, Irvine, CA).

Study Design. Dogs were intravenously injected with 300 units/kg of heparin. Ten minutes later, heparinase I (0.625, 1.25, 2.5 or 5 IU/kg), the vehicle of heparinase, or protamine (3.9 mg/kg, equivalent to ~1.3 milligram per milligram heparin) was injected as a bolus (over 3–5 s) into the central circulation *via* the right atrial port of the pulmonary artery catheter (n = 4/group). The injection volume for the vehicle group was equivalent to the "heparinase 1 = 2.5 IU/kg" group. All agents were injected at room temperature. Although protamine is normally infused over 5–45 min in clinical practice, protamine was injected as a bolus in this study to compare protamine and heparinase I under identical experimental conditions and to make evident any side effects of either drug. Hemodynamic variables were measured, and blood was sampled before and after the injections of heparin and the reversing agents. Immediately on sampling arterial blood (3 ml/sample), high range ACT was measured in duplicates by Hemochron (Model 400, International Technodyne, Edison, NJ), which uses Celite as the activator (Celite-

ACT), and by Hemotec (Medtronic-Hemotec, Englewood, CO), which uses kaolin as the activator (Kaolin-ACT). Both Celite- and Kaolin-ACT were concurrently examined to determine any potential activator-dependent effects.

Chemicals

Heparinase I (Neutralase; heparin lyase, E.C. 4.2.2.7) was purified from the bacterium *Flavobacterium heparinum* by IBEX Technologies. Heparinase I was supplied in sterile vials (0.5 ml) at 200 IU/ml. An international unit is the amount of heparinase I required to cleave 1 μmol heparin glycosidic linkages per min at 30°C (109 IU = 1 mg). For the canine study, heparinase I was diluted to 20 IU/ml by adding 4.5 ml sterile water (*i.e.*, the final volume was 5 ml) immediately before use. The vehicle of heparinase I contained 134 mM of sodium acetate and 10 mM of sodium phosphate, with *p*H adjusted to 7.0–7.3 using NaOH. Protamine (protamine sulfate injection, USP, lot 083214) was obtained from Elkins-Sinn (Cherry Hill, NJ). Each milliliter of protamine sulfate injection contains 10 mg protamine sulfate and 9 mg sodium chloride in sterile water. Heparin (heparin sodium injection from bovine lung, 1,000 units/ml, USP, Lot 515 JX) was obtained from Upjohn (Kalamazoo, MI). All agents were refrigerated at 4–8°C until use.

Calculation and Statistical Analysis

For the dog study, the combined baseline values of all variables (n = 24) were tested for normal distribution using the Kolmogorov-Smirnov test. For the ACT variable, if the combined basal data were normally distributed (suggesting animals were from the same population), the data were analyzed using analysis of variance, followed by Newman-Kuels test for pairwise comparison (CRUNCH, V. 4 Statistical Package, Oakland, CA). For hemodynamic variables, if the combined basal data were normally distributed, the data were analyzed using the Quade rank test with repeated measures. To confirm that the normality of the data did not affect the final statistical outcome, all data were also analyzed using the Quade rank test with repeated measures. The results of the additional statistical analysis were consistent with the original statistical analysis. For the *ex vivo* study, data were not analyzed for normality because of the small sample size, but using Quade rank test with repeated measures.

Outliers were defined as values greater than two standard deviations from a treatment group mean at any given time.

Results

Ex Vivo *Study*

The basal heparin concentration was 3.3 ± 0.3 units/ml (~0.033 mg/ml). The concentration-response curves with different incubation duration are shown in figure 1. The ACT decreased as the heparinase concentration in the sample increased, up to heparinase I concentrations ≥0.490 μg/ml, after which ACT values were maximally reduced and a plateau level was reached.

When the concentration-response curves at different incubation durations were compared, ACT values corresponding to the control samples were significantly higher ($P < 0.01$) than the ACT of all heparinase doses. The ACT (mean ± SD) for the controls was 516 ± 129 s, and that corresponding to a heparinase concentration

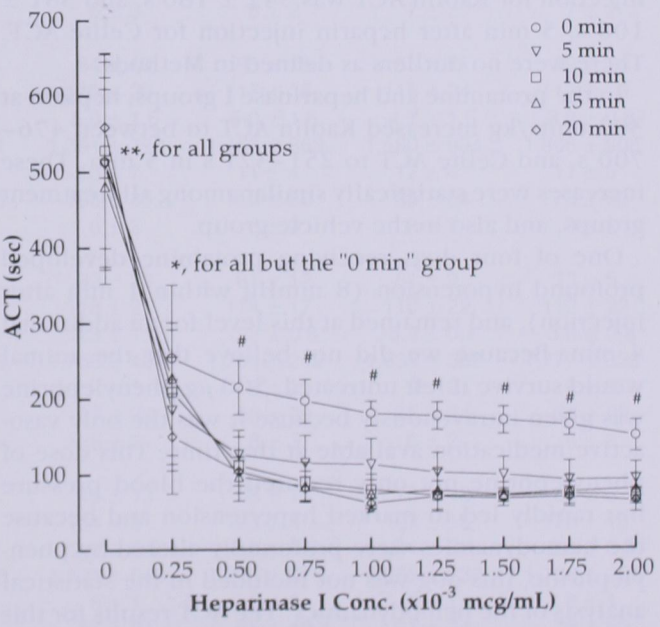

Fig. 1. Activated clotting time (ACT) vs. heparinase I (Neutralase) concentration determined after incubation at 37°C for 0 (activated clotting time measured immediately after heparinase I treatment), 5, 10, 15, or 20 min. Results are presented as mean ± SD, and n = 4/group. *$P < 0.05$, **$P < 0.01$ compared to activated clotting time values corresponding to the higher concentrations of heparinase I in this same curve; #$P < 0.05$, compared to equiconcentration values with 5–20 min of incubation.

of 0.245 μg/ml was 209 ± 90 s. Once the heparinase concentration was ≥0.490 μg/ml, there were no statistically significant differences in the ACTs obtained, whether the incubation time was 5, 10, 15, or 20 min. The ACT values (mean ± SD of ACT values corresponding to heparinase I concentrations at or above 0.490 μg/ml) were 105 ± 31 s for 5 min incubation, 81 ± 29 s for 10 min incubation, 80 ± 23 s for 15 min incubation, and 74 ± 19 s for 20 min incubation. All of these ACT values was significantly lower ($P < 0.05$) than that without any incubation (179 ± 30 s).

The minimum effective concentration of heparinase I was found to be 0.490 μg/ml for all concentration-response curves.

Canine Study

Activated Clotting Times. The baseline Kaolin and Celite ACT values were statistically similar among the six treatment groups (table 1). In the vehicle (control) group, heparin at 300 units/kg significantly increased both Kaolin and Celite ACT for at least 130 min (fig. 2). The peak increase, occurring at 5 min after heparin injection for Kaolin ACT was 542 ± 180 s, and 381 ± 108 s, 5 min after heparin injection for Celite ACT. There were no outliers as defined in Methods.

In the protamine and heparinase I groups, heparin at 300 units/kg increased Kaolin ACT to between 476–700 s, and Celite ACT to 251–371 s in 5 min. These increases were statistically similar among all treatment groups, and also in the vehicle group.

One of four dogs receiving protamine developed profound hypotension (8 mmHg within 1 min after injection), and remained at this level for an additional 4 min. Because we did not believe that the animal would survive if left untreated, 500 μg phenylephrine was given intravenously because it was the only vasoactive medication available at that time. This dose of phenylephrine not only restored the blood pressure but rapidly led to marked hypertension and because the hemodynamics were profoundly altered by phenylephrine, this dog was not included in the statistical analysis of the hemodynamics. The ACT results for this dog were similar to that of the other dogs receiving protamine and these data were included in the analysis of this group. In all the four dogs receiving intravenous bolus injection of protamine at 3.9 mg/kg, the heparin-induced increases in ACT were reversed within 5 min (Kaolin- and Celite-ACT values of protamine treatment group not significantly different from control values at 5 min). Similarly, intravenous boluses of heparinase I at 5–41 μg/kg completely reversed heparin-induced increases in ACT in 5–10 min (Kaolin ACT values of all treatment groups not significantly different from control at 5 min; Celite ACT values of the 2.5 and 5 μg/kg group not significantly different from control at 5 min, and that of all groups not significantly different from control at 10 min).

Hemodynamics. There were no outliers as defined in Methods. The baseline CI, MAP, index of systemic vascular resistance, and heart rate values were found to be normally distributed and not statistically different among all treatment groups. Basal values for mean pulmonary artery pressure, pulmonary artery occlusion pressure, and index of pulmonary vascular resistance were not found to be normally distributed. A Kruskal-Wallis analysis of variance indicated that the baseline values of the pulmonary parameters were not statistically different among treatment groups.

Figure 3 shows the change in CI and MAP of all the treatment groups. The bolus injection of the vehicle of heparinase (control) or of heparinase I at doses of 2.5 or 5.0 IU/kg produced a small decrease of the mean arterial pressure that was not statistically significant. The injection of heparinase I at doses of 0.625 and 1.25 IU/kg did not produce any hemodynamic effects. Protamine significantly decreased CI and MAP for 5–15 min when compared to the baseline value (fig. 3) and increased the mean pulmonary artery pressure and index of pulmonary vascular resistance.

Discussion

The *in vitro* study showed that the minimum effective concentration of heparinase I was 0.490 μg/ml. Based on the minimum effective concentration, the "heparin/heparinase I" ratio was found to be ~1/0.014. In a previous study using the identical preparation and design, we found that the ratio for protamine was ~1/1.3, and the ratio for platelet factor-4 (PF-4) was ~1/2.5.[6] The difference in the potency of these three reversing agents may be owing to the difference in their mechanisms of action: heparinase I neutralizes heparin by catalytically cleaving heparin,[7,8] whereas protamine and PF-4 stoichiometrically interact with heparin.[1]

The *in vitro* study also showed that the degradation of heparin by heparinase I can be enhanced by incubation at 37°C for 5 min. However, additional incubation beyond 5 min did not provide any significant

Table 1. Hemodynamic Data for All Dogs

	Heparin	Reversal	R + 1 min	R + 5 min	R + 10 min	R + 15 min	R + 30 min
Control							
MAP	70 ± 2	71 ± 4	61 ± 3	63 ± 6	68 ± 5	67 ± 1	69 ± 10
HR	117 ± 14	116 ± 17	119 ± 16	119 ± 13	117 ± 11	112 ± 15	109 ± 8
CI	3.9 ± 1.6	4.0 ± 1.7	4.4 ± 1.7	4.0 ± 1.2	3.8 ± 1.3	3.7 ± 1.1	3.3 ± 0.8
SVRI	1,582 ± 657	1,619 ± 757	1,177 ± 468	1,321 ± 395	1,675 ± 493	1,459 ± 402	1,694 ± 541
PAP	8 ± 2	8 ± 1	8 ± 1	8 ± 2	7 ± 1	8 ± 1	7 ± 1
PVRI	94 ± 64	98 ± 50	82 ± 24	92 ± 26	91 ± 30	96 ± 35	103 ± 39
PAOP	4 ± 2	3 ± 1	4 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1
Heparinase 0.625 IU/kg							
MAP	78 ± 13	75 ± 18	77 ± 12	71 ± 13	71 ± 14	70 ± 9	65 ± 8
HR	118 ± 9	116 ± 11	116 ± 9	117 ± 10	1,147 ± 7	112 ± 7	105 ± 7
CI	3.6 ± 0.4	3.9 ± 0.9	3.9 ± 0.8	4.0 ± 0.9	3.9 ± 0.9	3.9 ± 1.0	3.2 ± 0.7
SVRI	1,651 ± 457	1,560 ± 820	1,575 ± 637	1,474 ± 747	1,487 ± 759	1,462 ± 683	1,560 ± 385
PAP	7 ± 3	8 ± 2	8 ± 2	7 ± 2	7 ± 2	7 ± 2	6 ± 1
PVRI	60 ± 46	55 ± 32	64 ± 39	55 ± 33	56 ± 35	52 ± 34	57 ± 23
PAOP	4 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1	4 ± 1
Heparinase 1.25 IU/kg							
MAP	79 ± 24	77 ± 21	75 ± 23	75 ± 21	77 ± 22	75 ± 29	77 ± 25
HR	103 ± 35	100 ± 31	101 ± 32	104 ± 33	104 ± 32	103 ± 32	99 ± 31
CI	3.2 ± 0.7	3.7 ± 0.8	3.9 ± 1.5	3.2 ± 0.9	3.7 ± 1.1	3.6 ± 0.8	3.6 ± 1.2
SVRI	1,891 ± 763	1,624 ± 604	1,663 ± 487	1,777 ± 740	1,596 ± 478	1,592 ± 634	1,632 ± 613
PAP	6 ± 3	6 ± 3	7 ± 3	7 ± 3	7 ± 3	6 ± 3	8 ± 3
PVRI	39 ± 9	48 ± 14	44 ± 23	32 ± 3	49 ± 22	51 ± 14	59 ± 37
PAOP	6 ± 2	6 ± 2	6 ± 2	6 ± 3	6 ± 2	7 ± 2	7 ± 2
Heparinase 2.50 IU/kg							
MAP	98 ± 23	91 ± 21	81 ± 20	84 ± 19	85 ± 19	85 ± 21	89 ± 24
HR	124 ± 15	118 ± 23	119 ± 26	119 ± 26	117 ± 25	116 ± 24	113 ± 19
CI	4.4 ± 1.3	4.6 ± 1.6	4.6 ± 1.3	5.0 ± 1.6	5.0 ± 1.6	4.7 ± 1.6	3.9 ± 0.8
SVRI	1,634 ± 682	1,545 ± 771	1,316 ± 521	1,391 ± 798	1,463 ± 863	1,580 ± 994	1,886 ± 908
PAP	10 ± 3	11 ± 5	11 ± 5	13 ± 7	11 ± 4	12 ± 4	11 ± 5
PVRI	84 ± 9	86 ± 59	118 ± 105	108 ± 85	110 ± 86	86 ± 64	73 ± 80
PAOP	7 ± 3	8 ± 2	7 ± 2	8 ± 3	6 ± 3	8 ± 2	8 ± 2
Heparinase 5.0 IU/kg							
MAP	89 ± 15	84 ± 11	71 ± 16	82 ± 13	81 ± 12	81 ± 9	73 ± 5
HR	128 ± 28	124 ± 29	119 ± 23	118 ± 21	114 ± 17	110 ± 22	112 ± 22
CI	3.2 ± 0.7	3.8 ± 1.0	3.8 ± 1.3	3.3 ± 1.0	3.2 ± 0.8	3.3 ± 0.7	3.0 ± 0.8
SVRI	2,065 ± 202	1,640 ± 188	1,094 ± 898	1,768 ± 401	1,794 ± 242	1,835 ± 362	1,777 ± 326
PAP	9 ± 3	9 ± 2	10 ± 2	9 ± 3	9 ± 2	9 ± 2	8 ± 3
PVRI	94 ± 37	66 ± 33	81 ± 37	67 ± 36	66 ± 29	75 ± 34	64 ± 23
PAOP	6 ± 2	6 ± 2	6 ± 2	6 ± 3	6 ± 3	6 ± 2	6 ± 3
Protamine							
MAP	71 ± 11	68 ± 8	45 ± 6	49 ± 13	58 ± 13	70 ± 13	64 ± 5
HR	126 ± 16	118 ± 19	140 ± 12	134 ± 3	123 ± 9	123 ± 15	128 ± 13
CI	4.1 ± 1.3	3.3 ± 0.4	2.7 ± 1.1	2.0 ± 0.6	2.2 ± 0.3	2.4 ± 0.5	2.6 ± 0.7
SVRI	1,364 ± 397	1,498 ± 143	1,201 ± 476	1,721 ± 648	1,821 ± 570	2,068 ± 232	1,786 ± 257
PAP	7 ± 2	7 ± 2	13 ± 1	8 ± 1	6 ± 1	8 ± 1	8 ± 1
PVRI	42 ± 17	56 ± 19	123 ± 72	57 ± 31	65 ± 48	91 ± 34	73 ± 40
PAOP	5 ± 2	5 ± 2	8 ± 4	6 ± 2	4 ± 2	5 ± 2	5 ± 1

Hemodynamic data (mean ± SD) are given for the different treatment groups at the times of heparin administration, administration of the reversal agent (R), and 1, 5, 10, 15, and 30 min later.

MAP = mean arterial pressure (mmHg); HR = heart rate (beats/min); CI = cardiac index ($L \cdot min \cdot m^2$); SVRI = systemic vascular resistance index ($dyne \cdot s \cdot cm^{-5} \cdot m^2$); PAP = pulmonary artery pressure (mmHg); PVRI = pulmonary vascular resistance index ($dyne \cdot s \cdot cm^{-5} \cdot m^2$); PAOP = pulmonary artery occlusion pressure (mmHg).

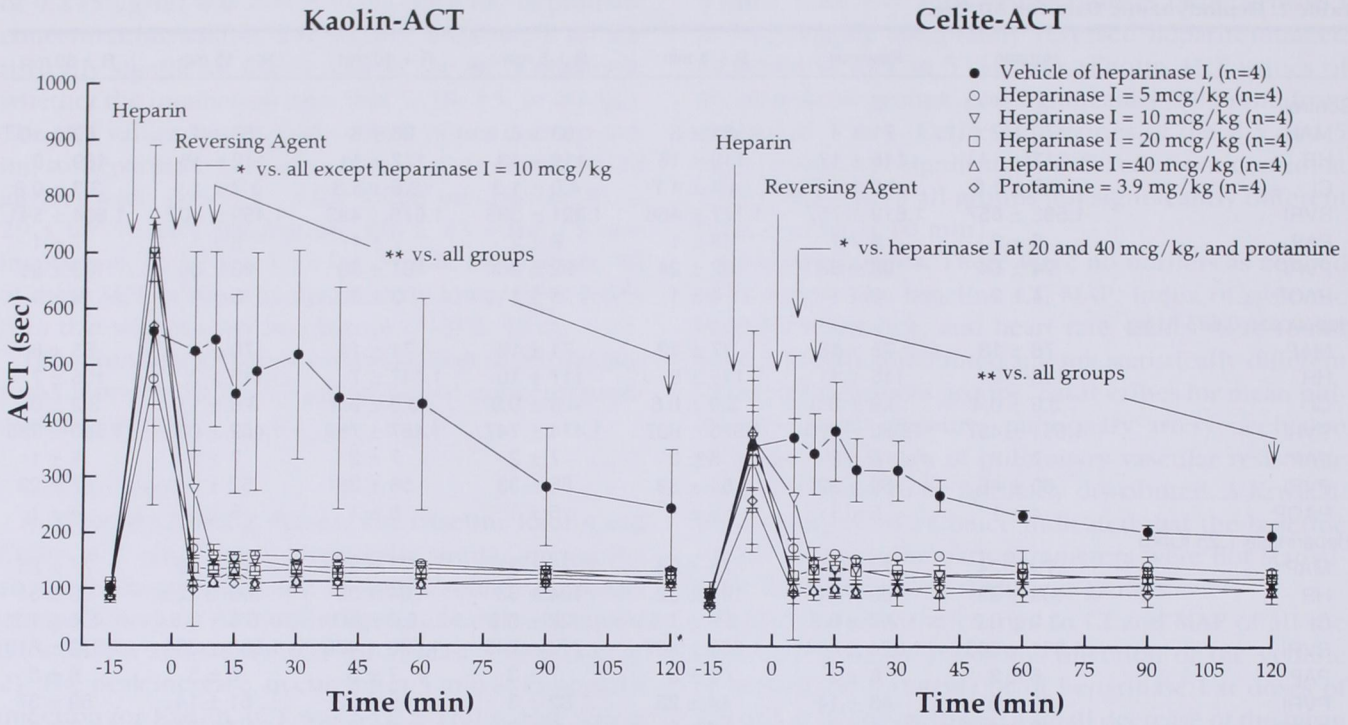

Fig. 2. Reversal of heparin-induced increases in Kaolin-(*left*) and Celite- (*right*) activated clotting time by protamine and various doses of heparinase I (Neutralase). Heparin (300 units/kg, intravenous) was injected at −10 min, whereas heparinase I, vehicle of heparinase I (0.125 ml/kg, a volume equivalent to 2.5 IU/kg) or protamine (3.9 mg/kg) was injected intravenously at 0 min. Results are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$, compared to the vehicle group. All heparinase I-treated and protamine-treated groups are statistically similar.

additional enhancement. The mechanism is unknown, but may be related to additional time to allow molecular interactions. Although incubation-induced enhancement may occur under *ex vivo* conditions, because the temperature of the blood can not be rapidly changed after discontinuation of CPB, such an enhancement is not applicable to *in vivo* conditions, which is the expected method of application of heparinase I.

The canine portion of this study showed that protamine (3.9 mg/kg) and heparinase I (5–41 μg/kg) reversed the anticoagulation effects of 300 units/kg heparin in 5–10 min in anesthetized dogs. This dose of heparin was used because it is an amount commonly used to produce anticoagulation for CPB in humans. It should be noted that there was a substantial difference between the dogs in the ACT prolongation as a result of heparin administration. Heparin-induced anticoagulation was reversed after 10 min, a period shown by Gravlee *et al.* to be adequate for measuring the anti-coagulation adequacy achieved with heparin before CPB.[9]

Heparinase could have been given as an infusion, rather than a bolus. However, we chose bolus administration because this form of delivery increases the chances of producing adverse hemodynamic effects with the drug and therefore such effects can be determined before the drug is used clinically. Our results indicate that bolus injection of heparinase I did not induce any statistically significant hemodynamic effects when compared to vehicle. There was a small, nonstatistically significant decrease in the MAP with bolus injection of the two higher doses of heparinase I and of the vehicle (control solution). Because the control solution contains the amount of solvent found in the 2.5 IU of heparinase group, this suggests that the solvent (sodium acetate and sodium phosphate) was responsible for this change, perhaps because of osmolarity. Protamine injected as a bolus induced systemic hypotension in all the dogs, which was profound in

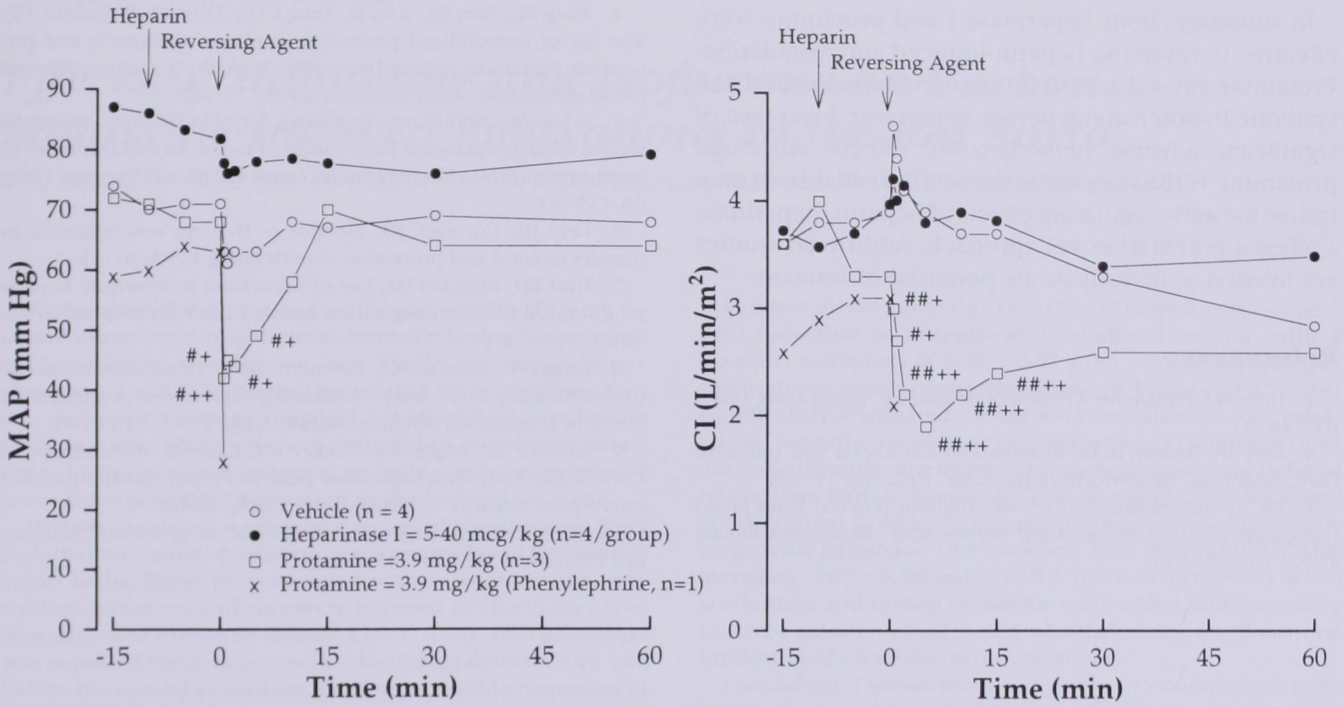

Fig. 3. Changes in mean arterial pressure (*left*) and cardiac index (*right*) before and after intravenous bolus injections of heparin at 300 units/kg and a reversing agent. The reversing agents were: heparinase I (Neutralase) at 0.625, 1.25, 2.5, or 5 IU/kg, vehicle (0.125 ml/kg, a volume equivalent to that of 2.5 IU/kg), and protamine (3.9 mg/kg). Results are presented as mean values. †$P < 0.05$, ‡$P < 0.01$, compared to the concurrent values of the vehicle group. For clarification, values at 60 min, which are statistically similar among all treatment groups, are not shown.

one of the four dogs 1 min after injection (MAP of 8 mmHg). This dog received intravenous phenylephrine because death appeared imminent without some intervention. Although phenylephrine is probably not the drug of choice in this situation, it was used because it was the only vasoactive drug available at the time. Indeed, it was surprising to see that the animal responded favorably, suggesting that systemic vasodilation rather than myocardial depression was a significant factor in the hypotension and that the increase in the pulmonary resistance could be overcome if coronary artery perfusion pressure was restored. Protamine also increased mean pulmonary artery pressure, pulmonary artery occlusion pressure, and index of pulmonary vascular resistance. The pulmonary hypertensive effect seen with the administration of protamine appeared to be caused by pulmonary vasoconstriction (reflected by increases in index of pulmonary vascular resistance). The hemodynamic effects of protamine observed in this study were consistent with those previously reported.[10]

Limitations

The animals were kept normothermic during this study. Because heparinase is an enzyme, it is also subject to temperature-dependent changes in its activity. It is therefore possible that heparinase will not be as effective in reversing heparin in the presence of hypothermia, but more studies will be needed to evaluate this possibility. In clinical practice, however, while heparin anticoagulation for cardiac surgery requiring cardiopulmonary bypass is often done under hypothermia, the patient is returned to normothermia before discontinuation of CPB. Reversal of heparin anticoagulation is therefore done at normothermia or close to normal temperatures.

Only one dose of heparin was used in this study. The dose selected, 300 units/kg, is a commonly used one for CPB anticoagulation, although some centers use larger or smaller doses. The results obtained are limited to the dose used, and further studies may be needed to determine if the effectiveness of heparinase varies with different heparin doses.

In summary, both heparinase I and protamine were effective in reversing heparin-induced anticoagulation. Protamine caused severe pulmonary hypertension and systemic hypotension whereas heparinase I was free of significant adverse hemodynamic effects. Although protamine is the only agent currently available to neutralize the anticoagulating effects of heparin, heparinase I offers a potential novel approach. Additional studies are needed to investigate its potential in humans.

References

1. Horrow J: Protamine: A review of its toxicity. Anesth Analg 1985; 64:348–61

2. Levy JH: Anaphylactic Reactions in Anesthesia and Intensive Care: Stoneham, Butterworth-Heinemann, 1992, pp 113–8.

3. Ma X, Mohammad SF, Kim SW: Heparin removal from blood using poly(L-lysine) immobilized hollow fiber. Biotechnol Bioeng 1992; 40:530–536

4. Yang VC, Port FK, Kim JS, Teng CLC, Till GO, Wakefield TW: The use of immobilized protamine in removing heparin and preventing protamine-induced complications during extracorporeal blood circulation. ANESTHESIOLOGY 1991; 75:288–97

5. Schoen P, Lindhout T, Franssen J, Hemker HC: Low molecular weight heparin-catalyzed inactivation of factor Xa and thrombin by antithrombin III—effect of platelet factor 4. Thromb Haemost 1991; 66:435–41

6. Levy JH, Cormack JG, Morales A: Heparin neutralization by platelet factor 4 and protamine. Anesth Analg 1995; 81:35–7.

7. Hutt ED, Kingdon HS: Use of heparinase to eliminate heparin inhibition in routine coagulation assays. J Lab Clin Med 1972; 79: 1027–34

8. Baugh RF, Deemar KA, Zimmermann JJ: Heparinase in the Activated Clotting Time Assay: Monitoring heparin-independent alterations in coagulation function. Anesth Analg 1992; 74:201–5

9. Gravlee GP, Angert KC, Tucker WY, Case LD, Wallenhaupt SL, Cordell AR: Early anticoagulation peak and rapid distribution after intravenous heparin. ANESTHESIOLOGY 1988; 68:126–9

10. Jacques LB: A study of the toxicity of the protamine, salmine. Br J Pharmacol 1949; 4:135–44