Reappearance of Circulating Heparin in Whole Blood Heparin Concentration-Based Management Does Not Correlate With Postoperative Bleeding After Cardiac Surgery

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Objective: **The Hepcon Heparin Management System (HMS) facilitates administration of higher heparin and lower protamine doses, which may affect bleeding potential due to heparin rebound. The present study evaluated heparin rebound in patients for whom the Hepcon HMS was used to determine whether point-of-care tests detect residual heparin and residual heparin is associated with postoperative blood loss.**

Design: **Prospective study.**

Setting: **Tertiary care center affiliated with a university hospital.**

Participants: **Adults undergoing elective cardiac surgery requiring cardiopulmonary bypass.**

Interventions: **In blood samples obtained at baseline, at 2 minutes, and at 1, 2, 4, 6, and 24 hours after heparin neutralization, heparin concentrations were measured using an automated chromogenic assay. Activated coagulation time (ACT), activated partial thromboplastin time (APTT), and thromboelastometry 2 hours after heparin neutralization also were examined in the last 22 study patients enrolled.**

Measurements and Main Results: **All 31 patients had measurable heparin levels 2 hours after protamine administration; 22 patients exhibited a primary failure to reverse heparin after protamine administration, and 9 patients had measureable heparin levels 2 hours after complete heparin reversal (ie, heparin rebound). The thromboelastometric variable, INTEM-CT:HEPTEM-CT ratio, correlated with heparin concentration ($r = 0.72$), but ACT ($r = -0.12$), APTT ($r = 0.36$), and whole blood heparin concentration, determined using the Hepcon HMS, did not. Peak heparin concentration (0.18 ± 0.07 U/mL) at 4 hours was not correlated with mediastinal blood loss.**

Conclusion: **Circulating heparin detected by the chromogenic assay was too low to be clinically significant based on postoperative bleeding, although all 31 patients had residual heparin or heparin rebound at 2 hours after protamine administration with use of the Hepcon HMS.**

KEY WORDS: heparin rebound, hepcon/HMS system, cardiopulmonary bypass, thromboelastometry, bleeding

COAGULOPATHY AFTER CARDIOPULMONARY bypass (CPB) is caused by multiple factors, such as disturbed hemostatic function due to hemodilution, coagulation factor depletion, platelet dysfunction, and activation of the fibrinolytic system.[1] Heparin rebound after adequate heparin neutralization[2] also is thought to contribute to microvascular coagulopathy. The incidence of heparin rebound varies from 4% to almost 100% of patients,[3–5] based on variations in the heparin and protamine dosing strategy and the methods used to assess anticoagulant activity, and the definition of heparin rebound.

Although heparin management based on activated coagulation time (ACT) is still common, ACT is not sensitive enough to detect low heparin concentrations that might occur in the presence of protamine reversal, hemodilution, low platelet numbers, or even excess protamine.[6,7] On the other hand, the Hepcon Heparin Management System (HMS) Plus (Medtronic, Minneapolis, MN) provides whole blood heparin concentration measurements using a heparin dose-response (HDR) assay and automated protamine titration on an individual basis,[7,8] because heparin sensitivity and the heparin clearance rate, as well as the amount of protamine required for heparin reversal, vary considerably from patient to patient.[9] Whether the higher heparin levels used during CPB and the reduced dose of protamine necessary for heparin neutralization provided by the Hepcon HMS are related to heparin rebound, however, remains unclear.

The aim of the present study was to evaluate heparin rebound using heparin concentration-based heparin monitoring and to determine whether laboratory and point-of-care tests accurately reflect the presence of circulating heparin and whether residual circulating heparin is associated with postoperative bleeding.

METHODS

After obtaining institutional review board approval and written informed consent from each patient, 32 adult patients scheduled for elective cardiac surgery requiring CPB were studied prospectively. Exclusion criteria included a history of any known coagulopathies, liver dysfunction, reoperations, preoperative abnormal coagulation profiles (international normalized ratio ≥ 1.3, APTT > 33 sec), and exposure to heparin, warfarin, clopidogrel, or direct thrombin inhibitors in the preceding 14 days.

No attempt was made to standardize the anesthesia, as the standard practice varies widely among professionals. The Hepcon HMS was used for all patients, and heparin (LEO Pharma, Ballerup, Denmark) was administered based on the results. A kaolin ACT of 480 seconds was determined to be the target value for the HDR on the Hepcon HMS. Because the calculation of the volume of a patient based on the body surface area is approximate, however, a 3,000 to 5,000 IU loading dose of heparin was added to the CPB pump prime.

All patients underwent normothermic CPB using a membrane oxygenator and biocompatible circuits (Capiox, RX-15 or 25; Terumo Corporation, Tokyo, Japan). Depending on the size of the patient, the extracorporeal circuit was primed with 550 mL of sodium bicarbonate, 30 g of mannitol, and 5 mg of betamethasone per kilogram of body

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weight when the body surface area was <2.0 m^2 or 800 mL of bicarbonate solution when the body surface area was ≥ 2.0 m^2.

After CPB termination, heparin was reversed using protamine sulfate based on the Hepcon HMS results. The heparin was considered neutralized once the ACT returned to the baseline level, and additional doses of protamine were not required based on the Hepcon HMS.

A cell-saver collection device (Cell Saver; Haemonetics Corporation, Braintree, MA) was used in all patients. Red blood cells were administered to patients with a hematocrit level of less than 20% during CPB or 30% thereafter. Allogeneic blood products were transfused based on visual assessment of empirical microvascular bleeding.

Blood samples (4 mL) were drawn via an indwelling arterial catheter after discarding approximately 6 deadspace volumes of the catheter and were collected immediately into 3.2% sodium citrate tubes (Venoject II, Terumo Corporation, Tokyo, Japan). Samples were obtained at the following times: (1) at baseline after the induction of anesthesia; (2) at 2 minutes after heparin neutralization using protamine sulfate; and at (3) 1 hour, (4) 2 hours, (5) 4 hours, (6) 6 hours, and (7) 24 hours after the end of the protamine infusion. Blood was centrifuged at 2,000 $\times g$ for 20 minutes to obtain platelet-poor plasma for measurement of the heparin concentration. The plasma heparin levels were measured in the laboratory using an automated chromogenic assay. In this assay, heparin is analyzed as a heparin-antithrombin complex after the addition of purified human antithrombin to the plasma sample. An excess of factor Xa then was added to the sample and neutralized by the heparin-antithrombin complex. The residual Xa level was quantified using a synthetic chromogenic substrate (lower limit of detection by chromogenic assay, 0.04 U/mL). The authors defined heparin rebound when the plasma heparin concentration was more than 0.04 U/mL after complete heparin neutralization.

In the last 22 patients enrolled in the study, blood samples obtained at 2 hours after the end of protamine infusion also were used for analysis of the whole blood heparin concentration, ACT (hemochron 401; International Technidyne Corporation, Edison, NJ), and APTT, and to perform thromboelastometry (ROTEM®; Tem Innovations GmbH, Munich, Germany). ROTEM was performed using citrated whole blood and intrinsically activated tests (INTEM test: 20 μL of 0.2-M $CaCl_2$, 20 μL of thromboplastin-phospholipid and ellagic acid, 300 μL of blood; and the HEPTEM test: Addition of 10 μL heparinase). As the coagulation time (CT) values mainly depend on the concentrations of coagulation factors and their inhibitors, the CT values were measured using the INTEM and HEPTEM tests.

Additionally, the following demographic and surgical data were collected: Sex; body weight (kg); height (cm); age (years); CPB duration (min); aortic cross-clamp time (min); use of tranexamic acid (No. of patients); chest tube drainage (mL) at 1, 2, 4, 6, and 18 hours after intensive care unit admission (a measure of postoperative blood loss); and the use of blood products (RBCs, FFP, platelet concentrate) during the intraoperative or 18-hour postoperative period.

Serial heparin concentrations were compared with the values at baseline and at heparin neutralization using a repeated measures analysis of variance followed by a paired *t*-test with Holm's correction. Pearson's correlation coefficients were determined between heparin concentration at 4h and blood loss at 1, 2, 4, or 6 hours, or total blood loss, and heparin concentration at 2 hours and various laboratory tests (ACT, APTT, ROTEM) at 2 hours after protamine neutralization. A pilot study of the chromogenic assay for plasma heparin revealed that the standard deviation of heparin concentrations was approximately 0.1 U/L. In this case, the required number of samples was estimated to be greater than 13, when alpha was set at 0.05/5 (five times comparison) and beta was set at 0.8 in a one-sided comparison. An unpaired t test was used to compare the patient data, perfusion time, heparin and protamine dose, and blood loss at 2 hours between incomplete reversed heparin and heparin rebound.

Table 1. Characteristics of Patients Enrolled in This Study

No. of patients	31
Sex	Male (22)/Female (9)
Age (yr)	71.1 ± 6.9
Weight (kg)	62.1 ± 13.4
Height (cm)	161.5 ± 11.0
CPB duration	122.4 ± 32.1
ACC time (min)	94.2 ± 25.7
Baseline ACT (sec)	146.7 ± 16.2
Mean CPB ACT (sec)	492.3 ± 52.0
After CPB ACT (sec)	125.3 ± 15.0
Total heparin dose ($U\times10^2$)	236.7 ± 54.7
Total heparin (U/kg)	381.0 ± 78.2
Total protamine dose (mg)	116.8 ± 30.7
Total protamine (mg/kg)	1.88 ± 0.47
Protamine : heparin ratio	0.56 ± 0.49
Tranexamic acid (No. of patients)	15
2 h chest drainage (mL)	143.8 ± 115.8
4 h chest drainage (mL)	275.2 ± 240.5
6 h chest drainage (mL)	340.6 ± 272.3
18 h chest drainage (mL)	489.4 ± 331.2

NOTE. Data are shown as the mean ± SD.

Abbreviations: ACC, aortic cross clamp; ACT, activated coagulation time; CPB, cardiopulmonary bypass.

Results are presented as the mean ± SD. The criterion for rejection of the null hypothesis was $p < 0.05$. All the statistical analyses, except statistical power analyses determined using G*Power 3.1, were performed using SPSS software (version 11.0; Chicago, IL).

RESULTS

One patient was excluded from the statistical analysis because of excessive bleeding requiring an additional dose of protamine; the remaining 31 patients were included in the analysis. None of the patients required surgical re-exploration postoperatively. The study patient characteristics are shown in Table 1. The surgical procedures performed in the 31 patients were as follows: 21 cases of valve replacement, 8 cases of both valve replacement and coronary artery bypass grafting, and 2 cases of aortic replacement. The mean dose of heparin, as estimated using the Hepcon HMS system, was 23,667 ± 5,466 U. None of the patients required additional heparin administration before instituting CPB due to failure to achieve their respective target ACT. This resulted in an ACT of 492 ± 52 seconds. The reversal dose of protamine sulfate was 122 ± 31 mg. The postoperative ACT (125 ± 15 sec) was significantly different from the baseline time of 147 ± 16 seconds ($p < 0.01$).

The mean and standard deviation of heparin concentrations measured at 2 minutes after heparin neutralization using protamine sulfate and at 1, 2, 4, and 6 hours, after the end of the protamine infusion using the automated chromogenic assay were 0.09 ± 0.05 U/mL, 0.11 ± 0.07 U/mL, 0.14 ± 0.07 U/mL, 0.18 ± 0.07 U/mL, and 0.14 ± 0.05 U/mL, respectively (Fig 1). Although heparin reversal based on the Hepcon HMS results was considered neutralization, 22 of the 31 patients exhibited residual heparin (0.09 ± 0.05 U/mL) after the initial protamine administration, representing unreversed