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A Phase III, Double-blind, Placebo-controlled, Multicenter Study on the Efficacy of Recombinant Human Antithrombin in Heparin-resistant Patients Scheduled to Undergo Cardiac Surgery Necessitating Cardiopulmonary Bypass

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Background: **The study evaluated the efficacy of recombinant human antithrombin (rhAT) for restoring heparin responsiveness in heparin resistant patients undergoing cardiac surgery.**

Methods: **This was a multicenter, randomized, double-blind, placebo-controlled study in heparin-resistant patients undergoing cardiac surgery with cardiopulmonary bypass. Heparin resistance was diagnosed when the activated clotting time was less than 480 s after 400 U/kg heparin. Fifty-four heparin-resistant patients were randomized. One cohort received 75 U/kg rhAT, and the other received normal saline. If the activated clotting time remained less than 480 s, this was considered treatment failure, and 2 units fresh frozen plasma was transfused. Patients were monitored for adverse events.**

Results: **Only 19% of patients in the rhAT group received fresh frozen plasma, compared with 81% of patients in the placebo group ($P < 0.001$). During their hospitalization, 48% of patients in the rhAT group received fresh frozen plasma, compared with 85% of patients in the placebo group ($P = 0.009$). Patients in the placebo group required higher heparin doses ($P < 0.005$) for anticoagulation. There was no increase in serious adverse events associated with rhAT. There was increased blood loss 12 h postoperatively ($P = 0.05$) with a trend toward increased 24-h bleeding in the rhAT group ($P = 0.06$). There was no difference between the groups in blood and platelet transfusions.**

Conclusion: **Treatment with 75 U/kg rhAT is effective in restoring heparin responsiveness and promoting therapeutic anticoagulation in the majority of heparin-resistant patients. Treating heparin-resistant patients with rhAT may decrease the requirement for heparin and fresh frozen plasma.**

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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EFFECTIVE anticoagulation is a prerequisite for the safe institution of cardiopulmonary bypass (CPB). By inducing a conformational change in the antithrombin tertiary structure, unfractionated heparin increases the affinity of antithrombin for thrombin approximately 1,000-fold. Antithrombin is a naturally occurring plasma protein that inhibits thrombin and factor Xa (and other circulating coagulation factors) and binds to heparan sulfate moieties on the vascular endothelium to help maintain homeostasis of the hemostatic system. Heparin's anticoagulant effect is closely tied to antithrombin activity. Accordingly, abnormally low antithrombin plasma activity may lead to an altered anticoagulant response to heparin. During CPB, therapeutic anticoagulation is usually monitored by the activated clotting time (ACT), a simple and rapid point-of-care test. The decision to initiate CPB after heparin administration hinges on attaining an adequate ACT. Limited data exist that define the optimal ACT for initiation of CPB,[1] but historically, values less than 300 s were associated with grossly visible clots in the bypass circuit. In a 1,000-member survey of the Society of Cardiovascular Anesthesiology and American Society of Extracorporeal Circulation, it was found that the target ACT used by 82% of responders was 400–480 s or greater, with an additional 4.5% targeting an even higher ACT.[2]

Heparin resistance can be defined as the failure to achieve the desired ACT after a standard dose of heparin (*i.e.*, 300–400 U/kg).[2] Failure to achieve an acceptable ACT for CPB is usually managed by additional heparin administration. Less frequently, an exogenous fresh frozen plasma (FFP) or a plasma-derived antithrombin concentrate is required to restore heparin responsiveness. Investigators have found the incidence of heparin resis-

tance among cardiac surgery patients to vary between 4% and 13%, depending on the target ACT and heparin dose required and on whether patients have recently received heparin.[3–9]

Lower antithrombin levels are associated with a decreased heparin dose response as measured by the ACT.[10] Antithrombin supplementation has been used to improve the ACT response to heparin[11–14] and is associated with better thrombin inhibition.[10] Initial studies suggest that recombinant human antithrombin (rhAT) is effective in restoring heparin responsiveness in patients with heparin resistance.[15] FFP is readily available at most institutions and has been effectively used as an exogenous source of antithrombin to restore heparin responsiveness.[16–18] However, FFP is not an innocuous intervention and carries the risk of several complications, including viral infections, acute lung injury, and allergic reactions.[19–22] To achieve adequate anticoagulation in patients with heparin resistance, high doses of heparin may be required during CPB,[23] and high heparin dose may increase the risk of postoperative bleeding.[24] The current study was therefore designed to assess the efficacy of rhAT for improving heparin responsiveness in heparin-resistant patients undergoing elective cardiac surgery involving CPB, thereby avoiding the need for escalating doses of heparin and FFP administration.

Materials and Methods

Review and written approval of the study protocol by the Independent Ethics Committees or Institutional Review Boards at all the participating study sites was obtained before initiation of the study. Investigators obtained written documentation that assured the willingness of each committee to comply with the Food and Drug Administration and the International Conference on Harmonization guidelines for Good Clinical Practice. Written informed consent was obtained from each enrolled participant.

Study Design

This multicenter, randomized, double-blind, placebo-controlled, rhAT efficacy study included patients at 14 US and European study centers undergoing elective cardiac surgery requiring CPB. The investigators and scientists at GTC Biotherapeutics (Framingham, Massachusetts) designed the study collaboratively. Data were pooled and sent to GTC Biotherapeutics for analysis. Investigators had open access to the data and were able to verify all of the analyses.

An rhAT dose of 75 U/kg was chosen in this study based on results obtained in a previous study[15] that established that single dosing with 75 U/kg rhAT or more resulted in plasma antithrombin levels approximating 100% activity that were maintained throughout CPB. FFP has been described as a source of antithrombin to facilitate heparin anticoagulation in heparin-resistant patients before or during CPB.[16–18] FFP was therefore proposed as a potential alternative to rhAT administration for the purposes of this study. Patients aged 18–85 yr, scheduled to undergo elective cardiac surgery requiring sternotomy and CPB, were studied. Patients were deemed to be heparin resistant and were eligible for inclusion in the study if they met the following criteria: (1) Patients whose ACT was less than 480 s after intravenous administration of 400 U/kg heparin were eligible. (2) In addition to the ACT criterion, patients had to have either a baseline heparin dose response slope of 80 s or less using the Hepcon® Heparin Dose Response cartridge (Medtronics, Minneapolis, MN) or they had to have been receiving intravenous heparin before surgery regardless of the heparin dose–response slope. Patients who had recently received or were receiving one or more of the following medications were excluded: warfarin (within 3 days); streptokinase; tissue plasminogen activator; abciximab, eptifibatide, or tirofiban; or clopidogrel. Also excluded were patients with preexisting coagulopathy defined as a history of bleeding problems or a laboratory history of bleeding disorder (*e.g.*, von Willebrand disease, platelet disorder). Patients who received antifibrinolytic agents were also excluded.

Heparin-resistant patients were randomly assigned to one of the two treatment groups. Randomization was computer generated. There was block randomization to prevent center bias. A blinded randomization code for the study population of 52 patients was generated by GTC Biotherapeutics' Biometrics Department. Each study site was given an initial assignment for 8 patients. Additional assignment sets were given to sites that enrolled 8 patients. Randomization assignment was only to those patients who met the criteria for heparin resistance. Each institution's pharmacist was the only person who had access to the blinded randomization code and was responsible for keeping the integrity of the blind and for the preparation of the study drug, which was either rhAT or saline.

One group received a single-bolus 75-U/kg intravenous injection of rhAT, and the other received a single-bolus intravenous injection of a normal saline placebo (fig. 1). If the ACT remained less than 480 s after the randomized bolus, this was defined as treatment failure, and 2 units FFP was transfused. Additional heparin was administered during CPB as guided by the automated protamine titration method (Hepcon®; Medtronics) and ACT values. Reversal of heparin with protamine after CPB was per institutional protocol.

Blood for measurement of hematologic parameters was obtained just before administration of the study drug (0 min), at 30 min after the initiation of CPB, and just before heparin reversal with protamine. These parameters included Kaolin ACT without heparinase and

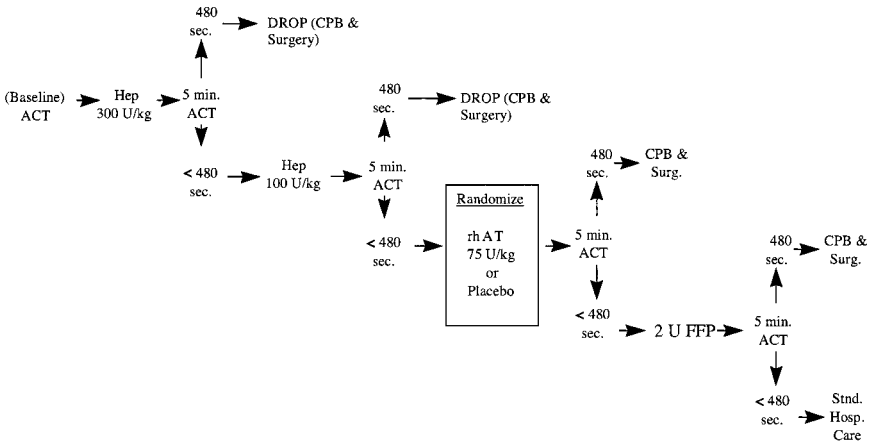

Fig. 1. Study design. Administration of heparin, study drug (recombinant human antithrombin [rhAT]), and fresh frozen plasma (FFP). ACT = activated clotting time; CPB = cardiopulmonary bypass; Hep = heparin; Hosp. = hospital; Stnd. = standard, Surg; = surgery.

plasma antithrombin activity levels. The ACT II® (Automated Coagulation Timer; Medtronics) device was used for measuring the ACT during the treatment period. ACTrac® (Medtronics), an electronic calibration device, was used in conjunction with the ACT II® device to insure that evaluation values using Hepcon® and study values using ACT II® were consistent between the two devices. Additional blood was obtained at baseline and 4 weeks postoperatively to monitor serum samples for antibody formation to rhAT.

Serum antibody testing (enzyme-linked immunosorbent assay [ELISA]) was performed at baseline, just before administration of the study drug, and again at approximately 4 weeks postoperatively. ELISA testing was used for initial screening for rhAT IgG antibody formation for patients in both the rhAT group and the placebo group. When ELISA test results were positive, a confirmatory radioimmunoprecipitation assay was performed. The sensitivity of the ELISA test had been confirmed before the study with experiments using rabbit serum with anti-human antithrombin antibodies. One hundred normal serum samples from Interstate Blood Bank had also been obtained to be used as controls. The specificity of the ELISA test had been confirmed with experiments using irrelevant proteins. The radioimmunoprecipitation assay also been subjected to extensive laboratory assessment and was shown to be both specific and reproducible in confirming the presence of antibody directed against rhAT. The limit of detection in the antibody assay was a 1:32,000 dilution of rabbit antisera.

All adverse events occurring from the time of study drug administration until hospital discharge and during the 4-week postoperative follow-up period were recorded. Blood and blood components transfused in the perioperative period were recorded. Postoperative blood loss through chest tube drainage was also recorded. Four weeks postoperatively, a blood sample for rhAT was obtained, and a postoperative review, including blinded review of medical and nursing records, for serious adverse events was performed.

The study's primary efficacy endpoint was whether rhAT restores heparin responsiveness leading to reduced transfusion of FFP and heparin administration. Patients who met the requirement for FFP but did not receive FFP were included in the analysis of patients who received FFP, intention-to-treat analysis. Secondary endpoints were comparisons between study groups of ACT values and plasma antithrombin activity levels in the peri-CPB period. All adverse events occurring from the time of study drug administration until hospital discharge and during the 4-week postoperative follow-up period were recorded.

Study Medications

Recombinant human antithrombin, manufactured by GTC Biotherapeutics, was supplied in clear glass, single-dose, 20-ml vials and was refrigerated at 2°–8°C until reconstitution. The institutional research pharmacist at each study site prepared all study materials and was the only person at the site who knew the treatment assignment. Normal saline, indistinguishable from the reconstituted rhAT at the designated concentration, served as the placebo control.

Statistics

Assuming a 10% dropout rate, treatment groups of 26 assessable patients provided greater than 80% power to detect a 40% absolute reduction (65% *vs.* 25%) in the proportion of patients requiring FFP.

The primary efficacy analysis was based on the intention-to-treat population, which included all randomized patients. The primary efficacy analysis, the difference between the proportions of patients requiring FFP in each treatment group, was assessed using the Barnard unconditional exact test. Differences in the treatment effect on the odds of requiring FFP were assessed using logistic regression. Odds ratios, comparing treatment groups, and 95% confidence intervals were calculated if indicated. The rank sum test was used for comparison of heparin between the groups. The Fisher exact test was used to compare the proportion of patients in each group requiring additional heparin doses. Differences

Table 1. Patient Characteristics by Treatment Group (Intention-to-treat Population)

Parameter	Statistic	Treatment Group: Placebo (n = 27)	Treatment Group: rhAT (n = 26)
Age, yr	Mean	66.0	67.6
	SD	10.9	9.5
	Minimum/maximum	48/86	49/80
Sex	Male (%)	18 (67)	23 (88)
	Female (%)	9 (33)	3 (12)
Race	White (%)	26 (96)	24 (92)
	African-American (%)	0	2 (8)
	Asian (%)	1 (4)	0
Weight, kg	Mean	74.5	78.4
	SD	15.6	12.0
	Minimum/maximum	50/103	55/101

rhAT = recombinant human antithrombin.

between the groups in blood loss and rate of bleeding were assessed with the Wilcoxon rank sum test.

The secondary efficacy endpoints, ACT values and plasma antithrombin activity, were examined at two times: 30 min after initiation of CPB and before protamine administration. Differences between the group means were assessed at each time point using analysis of variance.

The significance of shifts in safety parameters was evaluated within each group utilizing the McNemar test for binary variables and the generalized McNemar test for more than two categories. The difference between proportions of patients experiencing other safety-related events (rehospitalization, incidence of erythrocyte transfusion, platelet transfusion, additional blood product transfusion, duration of surgical intensive care unit stay, duration of hospitalization, 30-day incidence of rehospitalization, myocardial infarction, stroke, or death) were assessed using the Barnard unconditional exact test.

The statistical software used to analyze the data was SAS/STAT® (Cary, NC).

Results

Of 296 consented and enrolled patients, 54 were found to be heparin resistant and were randomly assigned to receive rhAT (n = 27) or placebo (n = 27). Four of these patients had been receiving heparin infusions preoperatively. Table 1 summarizes the patient characteristics.

In the intraoperative period during which FFP administration was guided by study protocol, 5 of 27 patients (19%) in the rhAT group received FFP, compared with 22 of 27 patients (81%) in the placebo group ($P < 0.001$). In the 24-h postoperative period during which FFP administration was guided by clinician discretion, 12 patients (44%) in the rhAT group received FFP, compared with 6 (22%) in the placebo group ($P = 0.15$).

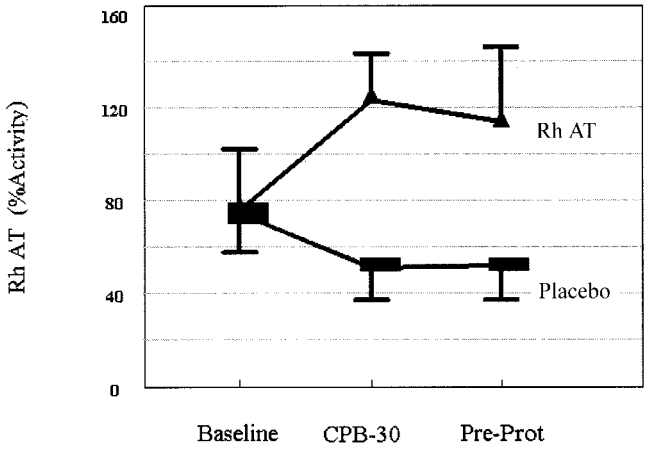

Fig. 2. Recombinant human antithrombin (rhAT) activity for each cohort at three time points around the cardiopulmonary bypass (CPB) period. Baseline = before institution of CPB; CPB-30 = 30 min after institution of CPB; Pre-Prot = CPB period just before protamine administration.

Transfusion of FFP during the entire hospitalization occurred in 37% fewer patients in the rhAT group (13 of 27 [48%]) compared with the placebo group (23 of 27 [85%]) ($P = 0.009$). There was no difference between groups in number of patients receiving erythrocyte (rhAT group, n = 23; placebo group, n = 27) or platelet transfusions (rhAT group, n = 7; placebo group, n = 7). These transfusions were according to clinician discretion.

The mean total amount of heparin given to patients during CPB was significantly greater ($P = 0.005$, rank sum test) among placebo patients (n = 22) who received FFP (6,890 U heparin/h) than among rhAT patients (n = 21) who did not receive FFP (3,884 U heparin/h). Twenty-one of 27 placebo patients (78%) required at least one additional dose of heparin between administration of study medication and heparin reversal with protamine. In contrast, only 12 of 26 rhAT-treated patients (46%) required at least one additional heparin dose during the same period ($P = 0.02$).

Hematologic Analysis

The mean antithrombin activity level at baseline did not differ between the rhAT and placebo groups and was above the 70% lower limit of normal physiologic activity (78% and 74%, respectively). However, there were statistically significant differences ($P < 0.001$) between the two groups with respect to plasma antithrombin activity 30 min after CPB initiation and before protamine reversal (fig. 2). In the rhAT treatment group, the mean plasma antithrombin activity level increased toward the upper limit of normal physiologic activity (130%) at both 30 min after initiation of CPB (122%) and before protamine reversal (113%). In contrast, the antithrombin activity levels among the placebo patients requiring FFP decreased further from baseline (74%) at both 30 min

Table 2. Descriptive Statistics for Activated Clotting Time (Seconds) for All Patients (Intention-to-treat Population)

	After Heparin*		5 min after Treatment		5 min after FFP		30 min on CPB		Before Protamine	
Statistic	rhAT	Placebo	rhAT	Placebo	rhAT	Placebo	rhAT	Placebo	rhAT	Placebo
n	26	27	25	26	3	20	24	26	25	27
Mean (± SD)	424 (± 38.5)	415 (± 56.7)	601 (± 177.6)	442 (± 106.1)	409 (± 23.2)	424 (± 92.5)	775 (± 205.1)	534 (± 126.2)	682 (± 234.9)	503 (± 179.1)
P value for group differences	0.472†		< 0.001‡		ND		< 0.001‡		0.003‡	

* 400 U/kg heparin was administered per protocol. † *P* value based on analysis of variance. ‡ *P* value based on rank sum test.

CPB = cardiopulmonary bypass; ND = not done; FFP = fresh frozen plasma; rhAT = recombinant human antithrombin.

after initiation of CPB (52%) and before protamine reversal (51%).

After heparin administration but before administration of study medication, mean ACT values were similar for both treatment groups (415 and 424 s, respectively; $P = 0.472$). However, 5 min after study drug administration, the rhAT group had a significantly higher mean ACT value compared with the placebo group (601 s and 442 s respectively; $P < 0.001$). Significantly higher mean ACT values were also observed in the rhAT group after 30 min on CPB and at the preprotamine time point (table 2).

The minimal effect of 2 units FFP on ACT is best illustrated by review of ACT values obtained 5 min after FFP administration in the 20 placebo patients who subsequently received FFP. After heparin and FFP administration, the mean ACT value for these 20 placebo patients was 424 s (415 s before FFP), which is notably less than the protocol standard that called for an ACT of 480 s or greater. Eight placebo patients had ACT values that were less than 360 s at 5 min after heparin and FFP administration; the lowest ACT recorded at this time point was 292 s.

Adverse Events

The incidence of adverse events that occurred in at least 10% of patients in either treatment group did not differ between study groups. The most frequently reported adverse events (≥ 20% incidence) in the rhAT treatment group were hemorrhage, atrial fibrillation, fever, postoperative pain, and pleural effusion. Those most frequently reported (≥ 20%) among placebo patients were postoperative pain and nausea. Apart from one stroke, there were no reports of thromboembolic events.

Two rhAT patients died postoperatively. Ten rhAT patients (37%) and seven placebo patients (26%) reported one or more serious adverse events. Only one serious adverse event, reported by an rhAT patient, was considered possibly related to study drug (hemorrhage). Serious adverse events associated with bleeding were reported in four rhAT patients (three hemorrhages, one coagulation disorder) and three placebo patients (three hemorrhages).

The first patient who died was a 70-yr-old woman with a history including cardiogenic shock after an inferior myocardial infarction and a previous stroke. She underwent triple vessel coronary artery bypass surgery. The first attempt to wean the patient from CPB was unsuccessful. She was successfully weaned from CPB on the second occasion on intravenous milrinone and norepinephrine and with the aid of an intraaortic balloon pump. She remained hemodynamically unstable and died several days later, with refractory heart failure.

The second patient who died was a 70-yr-old man with a history of severe angina pectoris. He underwent a scheduled three-vessel coronary artery bypass graft procedure, which was uncomplicated. The patient underwent extubation on the first postoperative day but was disorientated. A paresis of the left arm developed, and a brain scan showed bilateral cerebellar infarction, an infarction in the left frontotemporal region, and possibly in the right internal capsule, as well as hydrocephalus. The patient deteriorated rapidly and died on postoperative day 2. An autopsy revealed a previously undiagnosed severe obstructive atheromatous lesion of the basilar artery and the circle of Willis. There was atheromatous debris at the origin of the carotid arteries. Postoperative collateral history revealed that the patient had previously reported vertigo and had been diagnosed as having Ménière disease.

A placebo group patient experienced the only adverse event (postoperative pain) that was considered by study investigators to be definitely related to study medication. No patients in either treatment group experienced adverse events that were considered probably related to their study medication. Six rhAT patients and one placebo patient reported one adverse event each considered possibly related to study medication; in all cases, the adverse event was hemorrhage.

Additional Analysis of Bleeding and Transfusion

The incidence of adverse events associated with bleeding in each treatment group was closely reviewed. Hemorrhage (bleeding > 200 ml/h) occurred more frequently among rhAT patients (nine patients) than among placebo patients (five patients), but this trend was not statistically significant ($P = 0.35$). Eight of the nine reports of hemorrhage in the rhAT group occurred after protamine administration. Thrombocytopenia (platelets < 50×10^9/l) occurred in one placebo group patient. One

Table 3. Cumulative Total (First 24 h) and Rate (ml/h) of Chest Tube Drainage

Parameter	Treatment Group	n	Time Period and Between-group Differences: 12 h after Surgery	*P* Value	24 h after Surgery	*P* Value
Cumulative, ml	Placebo	27	756 ± 1,291	0.05	1,051 ± 611	0.06
	rhAT	27	1,290 ± 950		1,579 ± 1,048	
Rate, ml/h	Placebo	27	71	0.06	52	0.041
	rhAT	27	124		86	

rhAT = recombinant human antithrombin.

rhAT patient had a visible hematoma at the site of the skin incision over the sternum.

Mean total bleeding from chest tubes in the first 24 h postoperatively tended to be higher ($P = 0.06$) in patients who received rhAT (1,580 ± 1,048 ml) compared with placebo patients (1,216 ± 777 ml). However, the mean rate of chest tube drainage during the 24-h postoperative period was higher ($P = 0.041$) in the rhAT group (table 3).

Transfusion in each treatment group is summarized in table 4. Total perioperative administration of FFP was substantially less in the rhAT group compared with the placebo group (48% and 85%). The percentage of rhAT patients who received FFP within the 24-h postoperative period was twice (44%) that of placebo patients (22%). Packed erythrocyte and platelet transfusions were similar in the two groups.

AT Antibody Testing

Enzyme-linked immunosorbent assay results were negative for all patients in both groups at baseline and 4 weeks postoperatively. Seroconversion was not observed for any patient based on the ELISA results. Antibody testing at 4 weeks postoperatively was not done for five placebo patients and four rhAT patients.

Discussion

This study shows that rhAT effectively restores heparin responsiveness before CPB in the majority of patients with heparin resistance. The primary endpoint of this randomized, double-blind, placebo-controlled study was achieved. The proportion of patients requiring administration of 2 units FFP with the intention of achieving therapeutic anticoagulation, defined by an ACT of 480 s or greater, was significantly less ($P < 0.001$) in the rhAT-treated group (19%) compared with the placebo group (81%). There were three patients who received rhAT and FFP after heparin (400 U/kg) administration whose ACT remained less than 480 s. This suggests that some patients (11%) in the rhAT group had a heparin resistance mechanism that was not corrected with rhAT. Interestingly, rhAT restored heparin responsiveness even to some patients with heparin resistance who had antithrombin activity within the normal range.

Table 4. Erythrocyte, Platelet, and Fresh Frozen Plasma Administration

Parameter	Treatment Group: rhAT (n = 27)	Placebo (n = 27)
Administration of packed erythrocytes, n (%)		
Total	23 (85)	27 (100)
Prebypass initiation	2 (7)	5 (19)
On bypass to preprotamine administration	4 (15)	4 (15)
Protamine administration to end of surgery	4 (15)	4 (15)
Within 24 h after surgery	20 (74)	20 (74)
Administration of platelets, n (%)		
Total	7 (26)	7 (26)
Prebypass initiation	1 (4)	0
On bypass to preprotamine administration	0	0
Protamine administration to end of surgery	2 (7)	2 (7)
Within 24 h after surgery	5 (19)	5 (19)
Administration of fresh frozen plasma, n (%)		
Total	13 (48)	23 (85)
Prebypass initiation	3 (11)	16 (59)
On bypass to preprotamine administration	1 (4)	5 (19)
Protamine administration to end of surgery	3 (11)	3 (11)
Within 24 h after surgery	12 (44)	6 (22)

Bypass = cardiopulmonary bypass; rhAT = recombinant human antithrombin.

Although heparin resistance may be related to either congenital or acquired antithrombin deficiency, there are other important non–antithrombin-related heparin resistance mechanisms. Platelets, fibrin, vascular surfaces, and plasma proteins all modify the anticoagulant effect of heparin. Platelets decrease the anticoagulant effect of heparin by protecting surface factor Xa from inhibition by the heparin–antithrombin complex and by secreting platelet factor 4, a heparin-neutralizing protein.[25] Increased platelet count is also a possible cause of heparin resistance.[26] Fibrin limits the anticoagulant effect of heparin by protecting fibrin-bound thrombin from inhibition by the heparin–antithrombin complex. Thrombin also binds to subendothelial matrix proteins, where it is protected from inhibition by heparin.[27] There are many other circulating proteins (*e.g.*, lactoferrin, histidine-rich glycoprotein, vitronectin),[28–32] which bind to heparin and prevent its amplifying effect on antithrombin. A recent study showed that in some patients who received preoperative heparin infusions, heparin resistance developed without an abnormally low antithrombin activity.[23] During CPB, however, the antithrombin activity decreased below normal.[23]

One of the criticisms of our study might be that the 18% incidence (54 of 296 patients) of heparin resistance was surprisingly high compared with the 4–13% incidence previously reported. One of the reasons for this may be that we set the bar for our target ACT at 480 s, which is at the upper end of the range (400–480 s) that would be acceptable to most clinicians for CPB.[2] There is no universally accepted definition for heparin resistance, and as a diagnosis, it falls on a continuum ranging from mild to severe. We chose a clinical definition for our study, and it is possible that several patients with mild heparin resistance were included. The incidence of heparin resistance is reportedly as high as 43% when patients have been receiving heparin infusions.[23] Four of our patients had been receiving preoperative heparin.

The concept that antithrombin deficiency, or possibly altered responsiveness to antithrombin,[23] is an important cause of heparin resistance in patients undergoing cardiac surgery, especially those receiving heparin preoperatively, was supported by the study data. Study patients had a mean baseline antithrombin activity of 76% (normal, 70–130%), with 43% of these patients having baseline antithrombin activities less than normal (70%). Furthermore, CPB-associated hemodilution induced a further decline in antithrombin activity to a mean of 51% activity in placebo patients. In this group, antithrombin activity remained abnormally low throughout CPB.

In contrast, administration of 75 U/kg rhAT restored the mean plasma antithrombin activity to greater than 110% activity despite hemodilution during CPB and maintained mean antithrombin activity within normal physiologic range throughout CPB. This finding supports the chosen dose of rhAT (75 U/kg). The large disparity in maximum antithrombin plasma activity levels between the two groups (fig. 2) demonstrates that administration of 2 units FFP is not effective for normalizing plasma antithrombin activity.

Administration of rhAT at a dose of 75 U/kg was well tolerated by heparin-resistant patients undergoing CPB. There was no difference in the incidence of serious adverse events or the incidence of clinically significant abnormal laboratory parameters between the two treatment groups. Furthermore, rhAT antibodies did not develop in any rhAT-treated patients. It is possible that sites differed in the extent to which they reported adverse events. Investigators, however, were blinded as to group allocation and this decreases the likelihood of subjective reporting leading to bias in favor of one of the groups. There was also blinded review of medical and nursing charts to detect unreported adverse events.

Events surrounding the two patients who died in the rhAT group suggest that their deaths were attributable to comorbidities, which were heart failure and cerebrovascular atheromatous disease. It is unlikely that rhAT was implicated. The interpretation of the hemorrhage-related adverse events considered to have some relation to rhAT administration is of particular interest but is confounded by the multiple hemorrhage etiologies in the heart surgery and CPB setting. Excessive bleeding in rhAT-treated patients occurred after protamine administration. It is possible that a heparin–protamine mismatch may have contributed to increased postoperative bleeding in the rhAT group. With achieving near-normal postoperative antithrombin concentrations in rhAT-treated patients, low concentrations of residual heparin may have provided a more profound anticoagulant effect. Therefore, it may be important to administer a more accurately calculated protamine dose when rhAT is administered. Normal postoperative antithrombin levels in rhAT patients may have resulted in increased chest tube drainage if increased antithrombin activity amplified the effect of heparin rebound. Therefore, the increase in mean chest tube drainage among rhAT-treated patients is not totally unexpected in view of the lack of provisions to monitor and reduce heparin administration in the presence of normalized antithrombin activity or, more importantly, to monitor and treat heparin rebound postoperatively.[29,33–35]

During CPB, blood and the artificial surface of the bypass circuit come into contact, providing a powerful stimulus to activate the hemostatic system. Subsequently, tissue factor and tissue plasminogen activator are retransfused to the patient *via* cardiotomy suction resulting in systemic activation of the hemostatic system.[2] Without anticoagulation, clots can form in the bypass circuit and may result in circuit occlusion or intracardiac thrombosis.[36–38] In addition, with subtherapeutic anticoagulation, hemostatic system activation *via* thrombin and plasmin results in depletion of factors and

platelets due to inadequate suppression of a disseminated intravascular coagulopathy-like consumptive state. To prevent these events, heparin is used to promote the action of antithrombin in the blood thereby reducing thrombin generation, thrombin activity, and fibrin generation outside the body and systemically. Therefore, heparin resistance may lead to inadequate anticoagulation during CPB and potentially lead to an increase in bleeding, thrombotic complications, or both.

Although gross clot formation in the extracorporeal circuit rarely occurs, activation of the hemostatic system still has important clinical implications. CPB activation of the hemostatic system has been implicated in the generation of cerebral microemboli, one of the proposed causes of stroke, and neurologic deficit often observed after CPB.[39] Similarly, intraoperative coronary,[40–42] pulmonary,[43,44] intracardiac,[45] and CPB circuit[46] thromboses have all been described during cardiac surgery. Postoperative bleeding may result from platelet and labile coagulation factor consumption secondary to thrombin activity during CPB. Previous studies have demonstrated that more pronounced anticoagulation with heparin can reduce transfusion requirements[3] and preserve the hemostatic system.[46] Several studies have demonstrated that antithrombin supplementation can attenuate thrombin and fibrinolytic activity.[15] This suppression of thrombin and fibrinolytic activity, both of which usually increase during CPB, may represent better inhibition of hemostatic activation than that achieved with heparin alone.[15]

This is the first randomized, double-blinded, placebo-controlled trial that has demonstrated that rhAT is effective in the management of heparin resistance in patients undergoing cardiac surgery with CPB. Heparin resistance was caused by relative antithrombin deficiency in the majority of cases because the ACT was prolonged to the therapeutic range (> 480 s) in 81% of patients after rhAT administration. Administration of 75 U/kg rhAT restored antithrombin activity to within the normal range, which led to improved heparin responsiveness, decreased heparin requirements, adequate anticoagulation for CPB, and avoidance of FFP administration in the majority of cases. In contrast, antithrombin activity declined further from baseline in patients in the placebo group, who required FFP treatment and additional heparin administration to obtain adequate anticoagulation before CPB initiation. Two units FFP did not restore antithrombin activity to the normal range. Clinically, these findings may translate into a reduced risk of extracorporeal circuit or intracardiac thrombosis and postoperative bleeding due to the consumption of coagulation factors and platelets. Based on the increased chest tube drainage in rhAT-treated patients, clinicians should be aware of the risk of heparin rebound-induced bleeding when patients receive this agent. One of several point-of-care tests (*e.g.*, heparinase ACT, heparin-neutralized thrombin time) can be used to detect heparin rebound and direct protamine administration for reversal of heparin rebound-related bleeding. The trend toward increased bleeding with rhAT in this study raises a possible concern. Future research is necessary to determine whether there is increased bleeding associated with rhAT and whether this is related to the heparin rebound phenomenon. In summary, the current study demonstrated that 75 U/kg rhAT administered to heparin-resistant patients undergoing CPB effectively restores heparin responsiveness in the majority of cases. In addition, this prospective trial demonstrates that rhAT administration for management of heparin resistance decreases the heparin dose and the requirement for allogeneic plasma.

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