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Recombinant Human Transgenic Antithrombin in Cardiac Surgery

A Dose-finding Study

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Background: **Acquired antithrombin III (AT) deficiency may render heparin less effective during cardiac surgery and cardiopulmonary bypass (CPB). The authors examined the pharmacodynamics and optimal dose of recombinant human AT (rh-AT) needed to maintain normal AT activity during CPB, optimize the anticoagulant response to heparin, and attenuate excessive activation of the hemostatic system in patients undergoing coronary artery bypass grafting.**

Methods: **Thirty-six patients scheduled to undergo elective primary coronary artery bypass grafting and who had received heparin for 12 h or more before surgery were enrolled in the study. Ten cohorts of three patients each received rh-AT in doses of 10, 25, 50, 75, 100, 125, 175, or 200 U/kg, a cohort of six patients received 150 U/kg of rh-AT, and a control group of six patients received placebo.**

Results: **Antithrombin III activity exceeded 600 U/dl before CPB at the highest dose (200 U/kg). Doses of 75 U/kg rh-AT normalized AT activity to 100 U/dl during CPB. Activated clotting times during CPB were significantly ($P < 0.0001$) greater in patients who received rh-AT (844 ± 191 s) compared with placebo patients (531 ± 180 s). Significant ($P = 0.001$) inverse relations were observed between rh-AT dose and both fibrin monomer ($r = -0.51$) and D-dimer ($r = -0.51$) concentrations. No appreciable adverse events were observed with any rh-AT doses used in the study.**

Conclusions: **Supplementation of native AT with transgenically produced protein (rh-AT) in cardiac surgical patients was well tolerated and resulted in higher activated clotting times during CPB and decreased levels of fibrin monomer and D-dimer.**

HEREDITARY antithrombin III (AT) deficiency is seen in 1 of 2,000–5,000 individuals in the general population.[1] Acquired AT deficiency is a considerably more common disorder associated with a variety of pathologic conditions, including cardiac surgery. During cardiopulmonary bypass (CPB), AT activity frequently decreases to as low as 40–50 U/dl.[2–7] This abnormally low AT activity is observed in patients with hereditary deficiency and implicated in the pathogenesis of thrombotic episodes in these patients. AT deficiency may lessen the anticoagulant response to heparin, resulting in excessive activation of the hemostatic system during CPB[3,5,8] with subsequent consumption of labile coagulation factors and platelets[6] and excessive microvascular bleeding.[9] Excessive microvascular bleeding after cardiac surgery occurs in 5–16% of patients,[10,11] frequently requires transfusion of blood products, increases operative time and[9] need for exploration,[11,12] and overall increases the procedure-related morbidity and mortality.[11,13–16]

Patients undergoing cardiac surgery with CPB are also at increased risk for perioperative thromboembolic complications.[17–27] Despite the use of high doses of heparin during CPB, tissue injury during surgery and exposure of blood to the extracorporeal circuit may initiate excessive activation of the hemostatic system[28–30] and thrombin generation in a setting where the natural circulating anticoagulants such as AT and proteins C and S are commonly reduced.[2–7,31]

Antithrombin III concentrate derived from pooled human plasma is currently approved for patients with hereditary AT deficiency who require conventional heparin therapy.[32] However, clinical use of pasteurized AT concentrates has been limited by availability and concerns regarding the increased risk, albeit low, of infectious agent transmission by pooled products that involve large (*i.e.*, 20,000) donor exposures. Although AT concentrate is used in coronary artery bypass grafting procedures, it has not been approved for this and other causes of acquired AT deficiency in the United States. The development of transgenic, recombinant technology has facilitated the production of large quantities of pure proteins for potential use in humans. The potential advantages of transgenically produced recombinant proteins include a negligible risk of infectious agent transmission when compared with plasma-derived proteins, as well as potentially unlimited supply. Based on these considerations, we studied the effects of recombinant AT to determine the doses required to achieve a potential therapeutic concentration (*i.e.*, 100% activity) during CPB and to assess biologic activity of incrementally

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larger doses of AT with respect to enhanced suppression of hemostatic system activation during CPB.

Methods

Patient Enrollment and Recombinant Human Antithrombin III Dosing

Patients were enrolled after approval from the Institutional Human Studies Committee (Emory University Hospital, Atlanta, GA) and written informed consent was obtained. Thirty-six patients who were scheduled to undergo elective, primary coronary artery bypass grafting and who had received heparin for at least 12 h before surgery were eligible for this study. Exclusion criteria were previous coronary artery bypass grafting or valve surgery; unstable angina; preexisting hemostatic disorder, renal disease, or hepatic disease; recent or concurrent treatment with warfarin, streptokinase, tissue plasminogen activator, or abciximab (ReoPro®; Centocor Inc., Malvern, PA); preoperative blood or blood component transfusion; recent or active history of drug or alcohol abuse; known human immunodeficiency virus, hepatitis B, or hepatitis C infections; or known allergy or sensitivity to goat proteins.

This study was of a single-dose, open-label, dose-escalation design. Each of the following dosing cohorts of recombinant human (rh) AT consisted of three patients: 10, 25, 50, 75, 100, 125, 175, or 200 U/kg, respectively. The placebo and 150-U/kg cohorts each consisted of six patients. Patients were enrolled in the next higher-dose cohort after establishment of safety of the previous dose, which was assessed at least 48 h after the last patient within the previous cohort had completed therapy. The decision to advance to the next cohort was reviewed by a safety board composed of the principal investigator from the study site (or a designated representative) and a Medical Affairs representative from the study sponsor (Genzyme Transgenics Corporation, Boston, MA).

Study Procedures

Patients' medical history was recorded and a physical examination was performed within 30 days before surgery. Study medication was reconstituted from a lyophilized vial preparation of rh-AT (7 U/mg specific activity) provided by Genzyme Transgenics Corp. After induction of general anesthesia and tracheal intubation, patients in each dosing cohort received continuous intravenous infusion of rh-AT over 30 min. Patients then received an initial dose of 300 U/kg of porcine intestinal, unfractionated heparin. During CPB, whole heparin concentrations and the activated clotting times (ACTs) were determined at 30-min intervals using a heparin–protamine titration system (Medtronic Perfusion Systems, Minneapolis, MN). Additional heparin was administered as needed to maintain whole blood heparin concentrations between 2.7 and 3.4 U/ml (there were no target ACTs). Coronary artery bypass grafting surgery was performed during moderate hypothermia to 32°C using either vein or artery grafts. After discontinuation of CPB, heparin was neutralized with protamine at a dose ratio of 1.3 mg protamine per milligram of heparin in plasma. The protamine dose was calculated using whole blood heparin concentration measurements performed at the end of CPB. Subsequently, a heparinase-ACT was measured every 2 h after protamine administration for a total of 8 h to detect possible heparin rebound.

Blood specimens were drawn from an indwelling arterial catheter at the following time points: before and 1 min after completion of rh-AT administration; 5 min after heparin bolus administration; 30, 60, and 90 min after initiation of CPB; before protamine administration; and 10 min, 2, 4, 6, 12, and 24 h after protamine administration. AT activity (Coatest Antithrombin; Pharmacia Hepar Inc., Franklin, OH) was measured in all blood specimens. Heparin concentrations (Stachrom Heparin; American Bioproducts, Parsippany, NJ) were measured before rh-AT, after heparin, during CPB every 30 min, and 6 h after protamine. Turbimetric platelet aggregation (platelet aggregometer; Chronolog Corp., Havertown, PA), using 2.4 μM adenosine diphosphate and 2.4 μg/ml collagen (Chronolog Corp.), and bleeding times were measured before rh-AT and 2 h after protamine. The following analyses were performed on all perisurgical samples and at 10 min and 2 and 6 h after protamine: celite ACT using the Hemochron system (International Technidyne, Edison, NJ), kaolin ACT with or without heparinase using the ACT II instrument (Medtronic Perfusion Systems, Minneapolis, MN), heparin concentration (Stockroom Heparin; American Bioproducts), prothrombin fragment 1.2 (Enzygnost F1+2 micro; Behring Diagnostics Inc., Westwood, MA), fibrin monomer (Coatest Fibrin Monomer; Pharmacia Hepar Inc.), D-dimer (Asserachrom D-Di; American Bioproducts), plasmin:antiplasmin complexes, (Enzygnost PAP micro; Behring Diagnostics Inc.), and platelet function and β-thromboglobulin (Asserachrom β-TG; American Bioproducts). Antibody titers were measured before rh-AT administration and again at least 4 weeks after surgery using a qualified indirect enzyme-linked immunosorbent assay developed by Genzyme's Immunology Department and confirmatory radioimmunoprecipitation assay. Based on statistical analysis of the distribution of 200 normal human serum samples, 98% of the population has a final enzyme-linked immunosorbent assay optical density reading less than 0.100. Samples were classified as within normal range or above normal range based on their optical density readings. All serum samples with optical density greater than 0.100 were considered above normal range. A patient with a serum sample within normal range before treatment and above normal range after treatment was considered seroconverted if

Table 1. Demographic and Operative Patient Data

	Placebo	rh AT
Age (yr)	60 ± 10	66 ± 8
Race (number of white patients)	6	30
Gender (number of females)	2	12
Body weight (kg)	82.5 ± 10.8	78.5 ± 15.6
Preoperative aspirin (n)	6	22
Coronary artery bypass grafting		
Mean number of vessels grafted	3.3 ± 1.0	3.3 ± 0.7
Number with LIMA graft	3	11
Cardiopulmonary bypass (min)	75.2 ± 20.4	89.8 ± 24.1

Data are expressed as number or mean ± SD.

rh AT = human antithrombin; LIMA = left internal mammary artery.

the above normal range was confirmed as a positive reaction with the confirmatory assay (radioimmunoprecipitation assay).

Hemodynamic variables (*e.g.*, systolic and diastolic blood pressure, pulse) were monitored before and after AT administration and 24 h after surgery. A postoperative clinical evaluation was obtained at least 1 month after surgery, during which a blood sample was obtained for rh-AT antibody screen. A 12-lead electrocardiograph was obtained before AT treatment (or before heparin in controls) and within 24–48 after surgery. Chest tube drainage was recorded after protamine administration at 10 min and at 2, 4, 6, 12, and 24 h after arrival in the intensive care unit.

Statistical Analyses

One-way analysis of variance was used to compare continuous variables between treatment cohorts, while the chi-square test was used to compare categoric variables between cohorts. Univariate linear regression was used to evaluate the potential linear relation between any two variables. A P value ≤ 0.05 was considered significant.

Results

The demographic and operative profiles of patients receiving placebo or rh-AT are compared in table 1. Overall, no clinically important safety concerns were discovered in this study. No differences in hemodynamic variables were found between patients receiving rh-AT or placebo.

Chest Tube Drainage, Donor Exposures, and Adverse Events

There were no dose-related trends in chest tube drainage or transfusion requirements (table 2). There was no difference ($P = 0.15$) for total donor exposures between the placebo (1.08 ± 1.6 U) *versus* rh-AT–treated patients (4.8 ± 4.8 U). In addition, there was less transfusion support for the rh-AT-treated patients intraoperatively (1.7 ± 1.8 U) *versus* postoperatively (3.1 ± 4.3 U), and

Table 2. Adverse Events in Placebo and rh AT Patients

Adverse Event	Placebo (n = 6) [n (%)]	rh AT (n = 30) [n (%)]
Postoperative pain	6 (100)	29 (96.7)
Back pain	1 (16.7)	3 (10.0)
Hypotension (cardiovascular)	1 (16.7)	7 (23.7)
Agitation	2 (33.3)	5 (16.7)
Pericardiatis	1 (16.7)	4 (13.3)
Tachycardia	3 (50)	3 (10.0)
Rigors	2 (33.3)	1 (3.3)
Confusion	1 (16.7)	1 (3.3)

rh AT = human antithrombin.

there was no significant difference ($P = 0.71$) in the cumulative blood loss between the placebo-treated (822 ± 329 ml) and rh-AT-treated (893 ± 440 ml) patients.

Table 3 summarizes the adverse event frequency in the 30 patients treated with rh-AT and 6 patients in the placebo group. No serious adverse events related to rh-AT administration were reported. One rh-AT–treated patient died. This patient was noted at surgery to have severe diffuse atheromatous disease with loose plaques and suffered an atheromatous embolic stroke during surgery.

Antibodies to Recombinant Human Antithrombin III

Enzyme-linked immunosorbent assay testing for circulating antibodies to rh-AT was positive in three patients: one patient in each 100-, 125-, and 150-U/kg dose groups. Because no antibodies were detected in any patients in higher dose groups (175 and 200 U/kg), there was no relation between dose and reactivity. Positivity (three patients) of enzyme-linked immunosorbent assay testing was not confirmed on radioimmunoprecipitation assay (negative radioimmunoprecipitation test); thus, none of the three patients seroconverted. One of the

Table 3. Total Blood Component Donor Exposures and Mediastinal Chest Tube Drainage in Patients Receiving Different Doses of rh AT (n = 30) or Placebo (n = 6)

Treatment Group	n	Total Blood Donor	Chest Tube Drainage
Placebo	3	1.8/2 (0–4)	822/945 (362–1,120)
rh AT (10 U/kg)	3	4.0/4 (3–5)	771/790 (495–1,029)
rh AT (25 U/kg)	3	4.3/2 (2–9)	992/820 (645–1,510)
rh AT (50 U/kg)	3	2.3/0 (0–7)	898/875 (720–1,100)
rh AT (75 U/kg)	3	3.0/3 (2–4)	497/450 (432–610)
rh AT (100 U/kg)	3	2.0/2 (1–3)	968/905 (795–1,205)
rh ATII (125 U/kg)	3	2.5/2.5 (1–4)	1,370/960 (640–2,510*)
rh AT (150 U/kg)	6	8.3/6 (2–20)	1,053/740 (485–2,275)
rh AT (175 U/kg)	3	4.3/5 (2–6)	907/967 (475–1,280)
rh AT (200 U/kg)	3	8.3/4 (2–19)	966/595 (520–1,783)

Values are expressed as mean and median (*i.e.*, mean/median) with ranges in parentheses.

* Patient documented with surgical bleeding.

rh AT = human antithrombin.

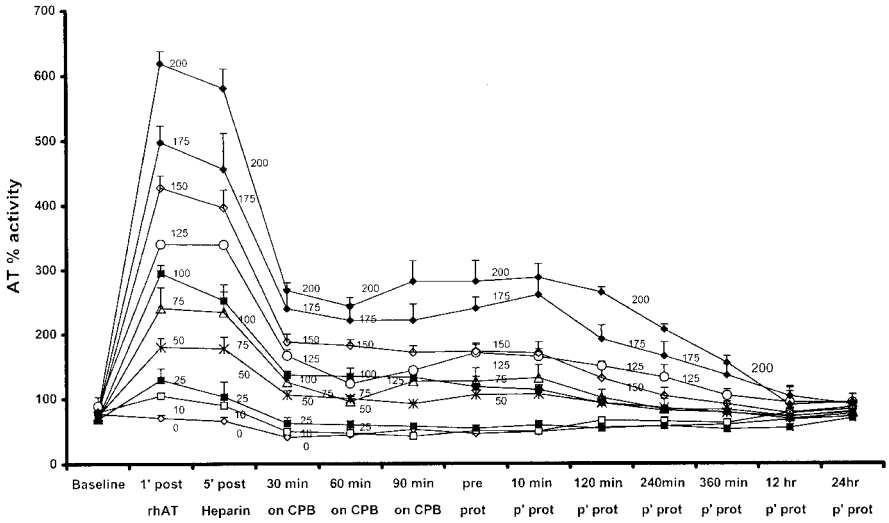

Fig. 1. Plasma antithrombin III (AT) activity concentrations after administration of recombinant human AT (rh-AT) using single doses of 0, 10, 25, 50, 75, 100, 125, 150, 175, and 200 U/kg during cardiac surgery with cardiopulmonary bypass (CPB). rh-AT was administered after intubation. Specimen collection times: Baseline = preadministration, 1 min after rh-AT injection, 5 min after heparin, every 30 min during CPB, before protamine, 10 min and 2, 4, 6, 12, and 24 h after CPB, and protamine reversal. Data are expressed as mean ± SE.

three patients showed reactivity (above normal range) before receiving rh-AT.

Antithrombin III Activity

Figure 1 shows the dose-dependent increase in plasma antithrombin activity after administration of each dose of rh-AT. With doses that exceeded 50 U/kg, AT plasma concentrations were maintained at greater than 100 U/dl throughout the CPB period. Furthermore, AT concentrations reached 600 U/dl and were maintained at approximately 250 U/dl throughout bypass after 200 U/kg rh-AT, the highest dose administered.

Thrombin Inhibition

The relation between fibrin monomer and rh-AT dose during and after CPB (n = 226) is shown in figure 2. A significant ($P < 0.0001$) inverse relation was observed between fibrin monomer concentrations and either rh-AT dose (fibrin monomer = −0.22 AT dose + 34; r = −0.51) or AT concentration (fibrin monomer = −0.25 AT + 44; r = −0.50) when rh-AT dose was less than 150 U/kg. The relation between AT activity and either mean D-dimer values for each dosing cohort or D-dimer concentrations during CPB (n = 121) is depicted in figure 3. A significant ($P = 0.001$) inverse relation was observed between D-dimer concentrations and either AT activity (D-dimer = −5.8 AT + 1,757; r = −0.49; fig. 3, top) or rh-AT dose (D-dimer = −6.5 AT + 1,586, r = −0.51; fig. 3, bottom). There were no significant differences in prothrombin fragment 1.2 concentrations between placebo and rh-AT groups at any time points studied ($P = 0.67$, r = 0.02; $P = 0.14$, r = 0.06); concentrations of prothrombin fragment 1.2 increased during CPB and were still elevated 6 h after administration of protamine. In addition, there were no significant differences between placebo and AT cohorts in concentrations of plasmin–antiplasmin ($P = 0.33$, r = 0.029; $P = 0.74$, r = 0.02) and β-thromboglobulin ($P = 0.33$, r = 0.03; $P = 0.74$, r = 0.02) concentrations measured over the course of CPB, nor at the preprotamine time point.

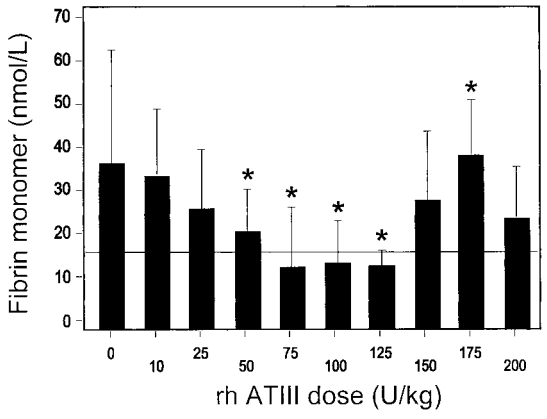

Fig. 2. Relation between activation of coagulation (fibrin monomer) and recombinant human antithrombin III (rh-AT) dose. Mean plasma concentrations of fibrin monomer for each dosing cohort are shown. The total number of specimens collected during and after cardiopulmonary bypass that were used for the analysis was 226. *$P < 0.05$ compared with placebo patients.

Platelet Aggregation and Bleeding Times

In all patients, platelet aggregation values after exposure to adenosine diphosphate and collagen decreased subsequent to protamine administration from the aggregation values observed at baseline. There was apparent effect of supplemental rh-AT (dose) on adenosine diphosphate–induced platelet aggregation ($P = 0.006$, r = 0.47) at the 2-h postprotamine time point, but no effect was seen in collagen-induced platelet aggregation.

There was no significant difference in bleeding time between placebo and rh-AT patients with respect to rh-AT concentration ($P = 0.62$, r = 0.12) or AT dose ($P = 0.66$, r = 0.15). In patients enrolled in the rh-AT dosing cohorts, the increase in median bleeding times from baseline to 2 h after protamine ranged from 0 to 8

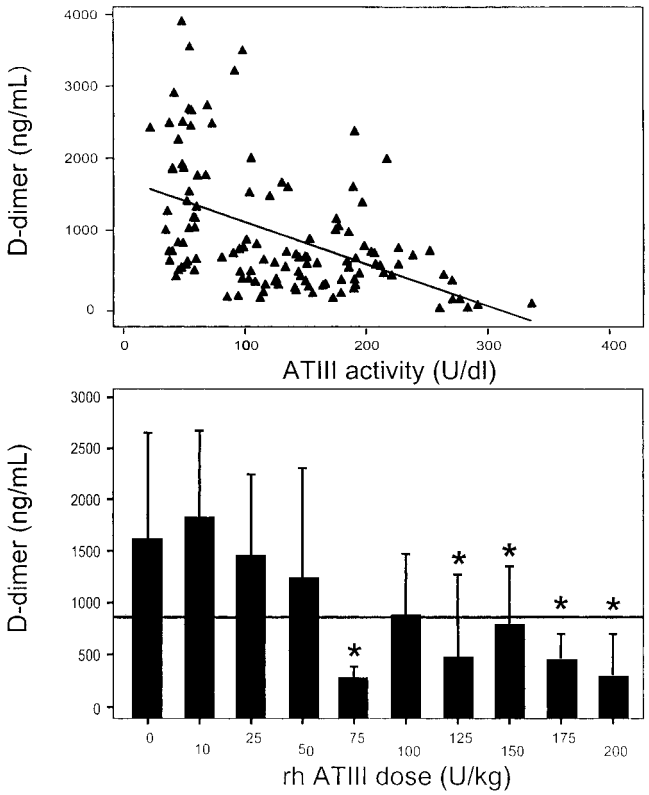

Fig. 3. Relation between D-dimer as markers of activation of coagulation (fibrin formation) and increased and recombinant human antithrombin III (rh-AT). (*Top*) The relation between individual plasma concentrations of D-dimer and rh-AT during several cardiopulmonary bypass time points is shown; a significant inverse relation was observed between D-dimer concentrations and rh-AT (D-dimer = −5.7 rh-AT + 1,749, r = −0.49, *P* = 0.001). (*Bottom*) Mean D-dimer values for each dosing cohort are shown. The total number of specimens collected at 15-min intervals during CPB used for analyses was 124. **P* < 0.05 compared with placebo patients.

min; in patients who received placebo, there was an approximately 4-min increase in the median bleeding time observed after protamine compared with baseline.

Activated Clotting Times, Anti-Xa, and Heparin Concentrations

Increased kaolin ACT values ($P < 0.0001$) were observed after systemic heparinization (*i.e.*, specimen collection time points 3–8) in specimens (n = 134) obtained from rh-AT–supplemented patients (844 ± 191 s) compared with ACT values obtained using specimens (n = 24) from placebo-treated patients (531 ± 180 s). This effect of rh-AT on ACT values appears to be independent of heparin because heparin concentrations (anti-Xa) measured in rh-AT–supplemented patients (5.0 ± 1.5 U/ml) were similar ($P = 0.14$) to those in placebo-treated patients (4.5 ± 1.6 U/ml, n = 24), and the effect of rh-AT on ACT values persisted even when heparin concentrations were included as a covariate in the analysis during CPB. Anti-Xa measurements at baseline, 5 min after heparin, and at CPB were comparable between the placebo and rh-AT groups: 0.38 *versus* 0.23 IU/ml (range, 0.15–0.41 IU/ml), 6.43 *versus* 6.67 IU/ml (range, 5.89–9.06 IU/ml), and 3.89 *versus* 5.07 IU/ml (range, 3.83–5.32 IU/ml), respectively. Likewise, heparin concentrations were 3.4 (placebo group) *versus* 4.0 IU/ml (rh-AT group) at 5 min after heparin and 2.7 *versus* 2.98 IU/ml during CPB.

Discussion

In this first study of a transgenic recombinant human protein in patients, rh-AT was shown to increase plasma AT concentrations without any serious adverse effects even when supraphysiologic doses were administered to supplement native AT in cardiac surgical patients. Furthermore, administration of rh-AT to 30 patients enrolled in current study resulted in no apparent differences in hemodynamic parameters, chest tube drainage, or transfusion requirements as compared with placebo-treated cohorts (table 2). Because pasteurized AT concentrates have been limited by availability, a new transgenic AT has been developed by modification of goat genome through transgenic technology.

Recombinant human AT is produced in milk of transgenic female goats during parturition.[33,34] The initial step in the development of a transgenic goat was creation of an expression vector that contained the sequence for the desired protein linked to the promoter sequence of a specific milk protein gene. This promoter sequence directs the production of the recombinant protein in the goat mammary gland on lactation. Specifically, the human AT cDNA coding for human AT was linked to the caprine β casein gene promoter.[35] The β casein gene is regulated in both a tissue and temporal fashion with maximal mammary gland expression occurring after parturition. The goat β casein gene was cloned from a Saanen goat genomic library and characterized in transgenic mice.[36] The caprine β casein gene was cloned as an 18.5-kilobase (kb) fragment in a λ EMBL3 vector. The human AT cDNA was obtained in plasmid pβAT6.[37] The sequence of cDNA is the same as that published by Bock *et al.*,[38] with the exception of the silent nucleotide changes at base pair 1050 (T-C), 1317 (C-T), and 1371 (A-G). The cDNA was engineered with an Xhol site at the 5' end by site-directed mutagenesis to allow excision of the cDNA as a 1.45-kb Xhol to Sa1l fragment.[35,36,38–40] The 6.2-kb β casein gene promoter was fused to human AT cDNA and 7.1-kb 3' flanking region of the β casein gene was added to the 3' end of the AT cDNA to help to stabilize expression levels.[33,35,38,39] The 14.75-kb transgene was excited from bacterial sequences and microinjected into preimplantation goat embryos, which were then transferred to the oviducts of surrogate mothers and carried to term.[33,34] A founder transgenic goat, a male, was identified for the presence of the transgene by analyzing genomic DNA from both a sample of ear tissue

Table 4. Pharmacokinetic Variables in Healthy Volunteers after a Single Intravenous Dose of rh AT

Variable	Value (SE)
V1	41.1 (3.7) ml/kg
Cl1	0.0383 (0.0287) ml/kg^{-1}
V2	74.3 (37.4) ml/kg
Cl2	0.0763 (0.0196) ml/kg^{-1}
C (endogenous AT)	0.914 (0.173) U/ml
T½ α	196 min
T½ β	2,568 min
Vd	115.4 ml/kg

rh AT = human antithrombin; SE = standard error; V1 = volume of the fast compartment; Cl1 = elimination clearance; V2 = volume of peripheral compartment; Cl2 = intercompartmental clearance; C (endogenous AT) = predicted baseline endogenous AT level; T½ α = distribution half-life; T½ β = elimination half-life; Vd = volume of distribution.

and blood by polymerase chain reaction[41] and Southern blot analysis.[38] This founder goat was bred to nontransgenic females and produced transgenic progeny. Once established in the first generation of transgenic animals, the transgene is transmitted as other genetic traits to future generations through traditional breeding, thus generating a production herd that supplies the raw rh-AT ready for purification.[34,42] A purification process that involves isolation of rh-AT from goat milk has been described with a yield greater than 50% and purity greater than 99.99%.[34,42] It involves clarification through a 500-kd tangential flow membrane filtration unit, heparin affinity, anion exchange, and hydrophobic interaction chromatography. This process removes endogenous goat AT present in the milk and other contaminating proteins.[42] Structurally, the recombinant human AT purified from goat milk is indistinguishable from plasma-derived AT with the exception of the glycosylation and carbohydrates.[34,42] Recombinant AT shows fourfold higher affinity for heparin than plasma-derived AT, which is attributed to the difference in glycosylation between recombinant and plasma-derived AT.[42] Recombinant and plasma-derived AT were shown to have equivalent activity in factor Xa and *in vitro* thrombin inhibition assays.[42] Lu *et al.*[43] investigated pharmacokinetics of a single intravenous dose of recombinant transgenic AT in healthy volunteers (table 4). The concentrations of AT after initial doses (50–200 U/kg) given intravenously over 30 min were best described by a weight-normalized two-compartment model.[43] Additional information regarding transgenically produced human AT has been previously reported.[44–46]

Although hereditary AT deficiency is rare,[1] acquired AT deficiency is common after noncardiac[47] and cardiac surgery.[2–7,48] Low AT concentrations during cardiac surgery are likely to develop because of preoperative use of heparin,[3–5] the effects of hemodilution,[3–5] and CPB-associated excessive hemostatic system activation.[6] AT concentrations commonly decrease to 40–50 U/dl activity[32] during CPB.[2–7] Native AT concentrations as low as 20–30 U/dl were observed during CPB in the current study in patients not receiving supplemental AT. Suprahysiologic AT concentrations were achieved at higher doses of rh-AT, reaching 600 U/dl immediately after the initial loading dose. Concentrations then declined secondary to hemodilution that occurs during initiation of CPB. Doses of rh-AT 75 U/kg normalized AT activity to greater than 100 U/dl during CPB, and AT concentrations remained elevated close to 100 U/dl on arrival in the intensive care unit in patients who received higher doses (*i.e.*, 150 U/kg). Thus, a single dose of AT maintains normal AT concentrations to cover the clinically important period of CPB during which excessive hemostatic system activation may occur and can lead to bleeding or thrombotic complications.

Anticoagulation is used during cardiac surgery to prevent thrombosis of the extracorporeal circuit and to minimize excessive CPB-related activation of the hemostatic system. Unfractionated heparin is routinely used because it is immediately effective, rapidly reversible, generally well tolerated, and inexpensive.[49] The heparin anticoagulant effect is predominately mediated by binding of heparin to AT, requiring a specific pentasaccharide sequence,[50] and subsequent irreversible complex formation and inactivation by AT of several activated coagulation factors, in particular, factors IIa and Xa.[51] Decreased heparin anticoagulant response or heparin resistance is associated with AT deficiency.[52] This has led to the use of either fresh frozen plasma[53] or human plasma-derived AT concentrates (AT concentrates are not approved for this indication in the United States)[5,54] in patients that show appreciable resistance to heparin before initiation of CPB.[53] Administration of rh-AT in the current study improved heparin anticoagulant effect, as reflected by increased ACTs during heparin therapy when compared with placebo patients.

Detection and treatment of acquired AT deficiency may also be important with respect to potential reduction in perioperative bleeding and thromboembolic complications. CPB-related activation of the hemostatic system[28–30] can consume labile coagulation factors and platelets, which may lead to excessive bleeding (*i.e.*, in 5–16% of patients)[10,11] or reexploration (3–4%)[11,12] after cardiac surgery. Acquired AT deficiency may render heparin ineffective in suppressing thrombin generation–activity during CPB.[3,5,8] This is important because recent evidence indicates that inadequate heparin anticoagulation can result in excessive bleeding and transfusion requirements[55] secondary to consumption of coagulation factors and platelets.[6] Therefore, detection and treatment of AT deficiency may be important with respect to preservation of labile coagulation factors and platelets, which may reduce blood loss and need for transfusion. The importance of AT in suppressing activation of the hemostatic system is supported by data from several studies.[3,52] Hashimoto *et al.*[3] showed that

AT supplementation more effectively suppresses thrombin activity in pediatric patients who received AT concentrates. Despotis *et al.*[52] demonstrated that appreciable increases in markers of platelet activation and coagulation activation were only seen when AT concentration decreased to less than 60 U/dl.

Because maintenance of normal or elevated plasma AT concentrations during CPB can enhance suppression of the hemostatic system, the current study investigated the efficacy of increasing doses of transgenic rh-AT to suppress markers of hemostatic activation. The findings of the current study indicate that normalizing AT concentrations with rh-AT during CPB can not only reduce thrombin activity (fibrin monomer), but can also reduce fibrinolytic activity (D-dimer) and reduce impairment of platelet function after CPB, which is thought to be the most important hemostatic defect after CPB.[56,57] Although this study did not show any reductions in excessive microvascular or perioperative thrombotic complications in patients receiving rh-AT, this could be because of the small sample size and low-risk population studied. Because we studied a small number of patients in each group, this study was not powered to prevent significant type II error; therefore, additional studies involving more patients will be necessary in the future to fully assess benefits of supplemental administration of rh-AT to cardiac surgical patients. Nevertheless, our data support the concept that rh-AT administration enhances heparin responsiveness and modulates activation of the hemostatic system.

Perioperative thrombotic complications such as deep venous thrombosis, pulmonary embolism, stroke (1.6–5.6%),[23–25] and perioperative myocardial infarction–injury (5–82%)[26,27] occur with cardiac surgery. Coronary,[17,18,22] pulmonary,[19,21] and intracardiac[20] thrombosis have also been described intraoperatively. Surgery and CPB result in excessive activation of the hemostatic system *via* both the intrinsic[28] and extrinsic[29,30] pathways, which leads to substantial thrombin generation and a prothrombotic state. The vascular endothelium and multiple circulating factors, including endothelium-derived relaxing factor, prostacyclin, tissue plasminogen activator, endothelium heparan sulfate, proteins C and S, and circulating AT normally inhibit intravascular thrombosis. However, normal circulating anticoagulants such as AT and proteins C and S are commonly reduced secondary to CPB-related consumption and hemodilution,[2–7,31] which may lead to hypercoagulability in the perioperative cardiac surgical period and thrombotic complications. Because perioperative bleeding and thrombotic complications after cardiac surgery may be related to inadequate suppression of the hemostatic system during and after cardiac surgery, detection and treatment of acquired AT deficiency may have important clinical consequences.

This is the first reported use of a transgenic recombinant protein for therapeutic application. Reductions in AT concentrations that occur during heparin therapy and extracorporeal circulation represent an important clinical application for rh-AT. Our data indicate that administration of transgenic rh-AT to patients and volunteers shows no adverse effects, can improve heparin responsiveness at doses used for CPB, and potentially preserve the hemostatic system *via* enhanced anticoagulation during CPB. Because our study included only a small number of patients in each dosing cohort, further studies with larger number of patients are needed to determine the clinical usefulness of rh-AT with respect to management of heparin resistance, prevention of excessive bleeding, and blood conservation, as well as prevention of thromboembolic sequelae. Other potential therapeutic applications that warrant investigation include administration of rh-AT to patients with unstable angina on heparin therapy or in patients undergoing angioplasty or other arterial manipulations.

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