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Influence of High-dose Aprotinin on Anticoagulation, Heparin Requirement, and Celite- and Kaolin-Activated Clotting Time in Heparin-pretreated Patients Undergoing Open-Heart Surgery

A Double-blind, Placebo-controlled Study

W. Dietrich, M.D., G. Dilthey, M.D.,* M. Spannagl, M.D.,† M. Jochum, Ph.D.,‡ S. L. Braun, M.D.,§ J. A. Richter, M.D.‖*

Background: **Aprotinin causes a prolongation of the celite-activated clotting time (CACT), but not of the kaolin-activated clotting time (KACT). Therefore, concern has been raised regarding the reliability of CACT to monitor anticoagulation in the presence of aprotinin. The current study was designed to test the efficacy of aprotinin to improve anticoagulation, and to investigate whether the prolongation of CACT reflects true anticoagulation or is an *in vitro* artifact. To elucidate this antithrombotic effect of aprotinin, this study was done in patients prone to reduced intraoperative heparin sensitivity.**

Methods: **In a prospective, randomized, double-blind clinical trial, 30 male patients scheduled for elective primary coronary revascularization and treated with heparin for at least 10 days preoperatively, received either high-dose aprotinin (group A) or placebo (group C). The CACT and KACT were determined, but only CACT was used to control anticoagulation with heparin. Parameters of coagulation that are indicators of thrombin generation and activity (F_{1+2} prothrombin fragments, thrombin-antithrombin III complex, and fibrin monomers), parameters of fibrinolysis (D-dimers), aprotinin, and heparin plasma concentrations were measured. Postoperative blood loss and allogeneic blood transfused were recorded.**

Results: **Total heparin administered was 36,200 units (95% confidence interval: 31,400–41,000; group C) compared with 27,700 (25,500–29,800) units (group A; $P < 0.05$). Hemostatic activation during cardiopulmonary bypass (CPB) was significantly reduced in group A compared with group C. After 60 min of CPB, all parameters were significantly different ($P < 0.05$) between the groups (group C *vs.* group A): F_{1+2} prothrombin fragments, 9.7 (8.9–11.7) ng/ml *versus* 7.5 (6.2–8.6) ng/ml; thrombin-anti-thrombin III complex (TAT), 53 (42–68) ng/ml *versus* 29 (23–38) ng/ml; and fibrin monomers, 23 (12–43) ng/ml *versus* 8 (3–17) ng/ml. Fibrinolysis was also attenuated; D-dimers at the end of operation were 656 (396–1,089) and 2,710 (1,811–4,055) ng/ml for groups A and C, respectively ($P < 0.05$). The CACT 5 min after the onset of CPB was 552 (485–627) *versus* 869 (793–955) s for groups C and A, respectively ($P < 0.05$), whereas the KACT showed no differences between the groups (569 [481–675] *vs.* 614 [541–697] s for groups C and A, respectively; P = NS). The 24-h blood loss was 1,496 (1,125–1,995) *versus* 597 (448–794) ml for groups C and A, respectively ($P < 0.05$).**

Conclusions: **Aprotinin treatment in combination with heparin leads to less thrombin generation during CPB. Aprotinin has anticoagulant properties. Celite-activated ACT is reliable for monitoring anticoagulation in the presence of aprotinin, because the prolonged CACT in the aprotinin group reflects improved anticoagulation. Kaolin-activated ACT does not reflect this effect of aprotinin. (Key words: Arteries, coronary: thrombosis. Blood: aprotinin; fibrinolysis. Blood, coagulation: antithrombin III; prothrombin fragments; thrombin. Complications: bleeding. Monitoring: activated clotting time. Pharmacology: celite; heparin; kaolin. Surgery: cardiac.)**

THE protease inhibitor aprotinin reduces blood loss after cardiac surgery.[1–7] Activation of fibrinolysis[8] is at-

This article is accompanied by a Highlight. Please see this issue of ANESTHESIOLOGY, page 29A.

* Staff Anesthesiologist, Department of Anesthesiology, German Heart Center.

† Research Fellow, Department of Hematology, University Clinic.

‡ Assistant Professor, Department of Surgery, Division of Clinical Biochemistry, University Clinic.

§ Staff Clinical Chemist, Institute of Clinical Chemistry, German Heart Center.

‖ Chairman, Department of Anesthesiology, German Heart Center.

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Address reprint requests to Dr. Dietrich: Department of Anesthesiology, German Heart Center, Munich, Lothstraße 11, D-80335 Munich, Germany.

tenuated by aprotinin treatment and platelet function is better preserved than in control patients after cardiopulmonary bypass (CPB).[9,10] In addition, some evidence indicates that aprotinin also acts as an anticoagulant.[11,12] This anticoagulant effect of aprotinin may be produced by the inhibition of the contact phase of hemostasis *via* kallikrein inhibition and subsequent reduction of clotting activation.[13]

Aprotinin causes prolongation of celite-activated clotting time (CACT),[14] which is commonly used in cardiac surgery to determine the efficacy of heparin-induced anticoagulation. Recently, it was questioned whether an ACT employing celite as an activator in the presence of aprotinin may lead to inadequate heparin-induced anticoagulation with the potential for a hypercoagulable state and the risk of subsequent graft occlusion and myocardial infarction.[15] On the other hand, if kaolin is applied as an activator, the ACT (KACT) is not prolonged in the presence of aprotinin.[16] Therefore, the KACT was recommended to control anticoagulation in patients treated with aprotinin.[12]

Patients with heparin-induced anticoagulation for several days before operation are prone to develop reduced sensitivity to heparin.[17–19] Heparin by itself has no anticoagulant properties. It accelerates the action of the physiologic inhibitor antithrombin III (AT III). Diminished AT III activity often found in these patients is one mechanism for poor sensitivity to heparin, which leads to increased activation of coagulation during CPB.[20] These patients with inadequate anticoagulation by heparin were chosen for the current study to investigate the influence of aprotinin on prothrombin activation. The underlying assumption was that a possible anticoagulant effect of aprotinin would be more evident under the conditions of increased activation of coagulation than in patients with normal heparin response, in whom the suppression of coagulation during CPB may be adequate solely by heparin.

We hypothesized that aprotinin contributes to heparin-induced anticoagulation by inhibiting prothrombin activation during CPB. The aims of the current study were: 1) to investigate the ability of aprotinin to inhibit thrombin generation during CPB in patients prone to inadequate anticoagulation by heparin alone, and 2) to determine whether the increase of CACT in the presence of aprotinin reflects true anticoagulation or is an *in vitro* artifact.

Materials and Methods

Thirty male patients, scheduled for elective primary coronary revascularization, gave informed consent to participate in this prospective, randomized, placebo-controlled study that had been approved by the local ethics committee. Inclusion criterion was preoperative treatment with heparin, either intravenously or subcutaneously, for at least 10 days before surgery. Patients with warfarin pretreatment were excluded from the study, but those receiving antiplatelet therapy until the operation were not.

Study Design

Patients were independently randomized, using a table of random numbers, to either the aprotinin group (group A) or the control group (group C). On the basis of a presumed difference of one standard deviation of fibrin monomers between aprotinin and control patients found in a previous study,[21] we determined that a sample size of 30 patients (15 in each group) would provide 80% power with a type I statistical error of 5%.

Protocol

Aprotinin and the respective placebo were provided by the manufacturer (Bayer AG, Leverkusen, Germany) in identical packages, each containing 14 bottles, that could only be identified by the random number. Each bottle of aprotinin contained 5×10^5 kallikrein inactivator units (KIU; = 70 mg) of aprotinin in 50 ml 0.9% saline solution, and the placebo bottles contained only saline. The dosage regimen of aprotinin was as follows: patients received a loading dose of 2×10^6 KIU aprotinin over a 15-min period at the start of surgery, followed by a continuous infusion of 5×10^5 KIU/h throughout the entire course of surgery. An additional bolus of 2×10^6 KIU was added to the prime of the heart-lung machine. Patients in group C received an equal volume of saline.

Anesthetic Technique

Anesthesia was induced by 0.02 mg/kg flunitrazepam and 10–20 μg/kg fentanyl. Neuromuscular blockade was achieved and maintained with 0.1 mg/kg pancuronium. To maintain anesthesia, 10–20 μg/kg fentanyl and 0.02 mg/kg flunitrazepam were given.

Control of Heparin-induced Anticoagulation

Blood was anticoagulated for CPB with 375 units/kg porcine mucosa heparin (La Roche, Basle, Switzerland) injected *via* the central venous catheter before aortic cannulation. Ten minutes later, the anticoagulation was monitored using CACT (Hemochron 800, Intern.

Technidine Corp., Edison, NJ) and KACT (Hemotech, Medtronic, Englewood, CO); each measurement was done in duplicate. According to our protocol, the effect of heparin was monitored by the CACT: if one of the two CACTs did not reach 400 s, an additional bolus of heparin of 125 units/kg was given.

These ACT measurements were performed every 30 min until the end of CPB. For data analysis, the lower value of the duplicate measurements of each method was used. Additionally, the mean ACT for all ACT measurements during CPB was calculated. Because, in high ranges, the ACT does not correlate with anticoagulation,[22] the measurement was discontinued if the CACT exceeded 1,000 s. The KACT was stopped automatically at 999 s by the analyzer. These data were used for analysis. After completion of CPB, residual heparin was neutralized by protamine chloride in a ratio of 1.5 mg/125 units of the initial heparin dose. Because the CACT in the presence of aprotinin[14] is still prolonged after protamine infusion, a KACT >140 s 15 min after protamine was an indication for repeat protamine (0.5 mg/kg).

Cardiopulmonary Bypass

The extracorporeal circuit consisted of a bubble oxygenator (High Flex D 700 S, Dideco, Mirandola, Italy), nonocclusive roller pumps, a cardiotomy reservoir (Dideco D 742, Mirandola, Italy), and polyvinyl tubing. The oxygenator was primed with 1,400 ml crystalloid solution, containing 5,000 units of heparin. Cardiopulmonary bypass was performed with moderate hypothermia of 30–32°C rectal temperature and a flow rate of 2.4 $l \cdot min^{-1} \cdot m^{-2}$. Myocardial preservation was achieved by infusion of 1,000 ml cold crystalloid cardioplegic solution (Bretschneider HTG, F. Köhler Chemie, Alsbach, Germany) into the aortic root after aortic cross clamping.

Transfusion Policy

The indication for allogeneic blood transfusion was a hematocrit of 30% or less after arriving at the ICU. Requirement of allogeneic blood and blood products was recorded during the whole hospital stay. Blood loss was recorded 2, 6, 12, and 24 h postoperatively and at the removal of the chest tubes. Blood loss greater than 300 ml/h during the first two postoperative hours or more than 200 ml/h during the subsequent time were indications for surgical reexploration. A cell separator (Haemonetics, Munich, Germany) was used to concentrate the remaining cellular contents of the oxygenator after termination of bypass. The hard shell reservoir of the heart-lung machine was employed for postoperative shed mediastinal blood collection. The shed mediastinal blood was retransfused up to 6 h after surgery if at least 250 ml blood had been collected.

Blood Samples

Blood samples were taken from the radial artery or, during CPB, from the port of the oxygenator at the following times: 1) after induction of anesthesia but before aprotinin infusion, 2) 10 min after the administration of heparin but before CPB, 3) 30 min after start of CPB, 4) 60 min after start of CPB, 5) at the end of CPB, and 6) at the end of operation. After the first 10 ml of blood were discarded, blood was drawn into EDTA tubes for measurement of hematocrit and platelet count, or into sodium-citrate tubes (1:9) for coagulation tests. The blood was centrifuged at 3,000*g* for 10 min at room temperature and the plasma was separated from the cellular components. All plasma samples were frozen immediately at −40°C in aliquots and thawed only before testing.

Plasma samples for β-thromboglobulin (βTG) were collected in precooled special sampling tubes (Kodak, Amersham, UK) and processed according to the manufacturer's instructions. Aprotinin plasma concentration was quantified by means of a competitive enzyme-linked immunosorbent assay.[23] The split products of the cross-linked fibrin were measured by two immunoassays, based on two independent monoclonal antibodies to D-dimers (Boehringer, Mannheim, Germany) and to fibrin (FbDP, Organon Teknika, Heidelberg, Germany). The complex of thrombin with antithrombin III (TAT), F_{1+2} prothrombin fragments, and platelet factor 4 (PF4) were determined by sandwich enzyme-linked immunosorbent assays (Behringwerke, Marburg, Germany). Fibrin monomers were measured by an immunoassay, using a monoclonal antibody against the N-terminal α chain of human fibrin[24] (Boehringer, Mannheim, Germany). Heparin plasma concentration was measured by a chromogenic substrate assay using the anti-IIa-inhibiting capacity (Instrumentation Laboratory, Kirchheim, Germany). Calibration of this test was performed by the same heparin preparation used in the patients during this study. The AT III activity was determined by a chromogenic substrate assay (Boehringer, Mannheim, Germany). Activated partial thromboplastin time (aPTT) was measured by standard methods preoperatively, 6 h postoperatively, and on the first postoperative day.

Data Analysis

Two-way ANOVA was used to analyze normally distributed data. When P was less than 0.05, *post hoc* comparisons were performed with Tukey's test. Parametric data are given as mean and 95% confidence intervals (CI 0.95). If Shapiro's test of normality revealed that data did not conform to a normal distribution (blood loss data, ACT, F_{1+2} prothrombin fragments, TAT, fibrin monomers, D-dimers, PF4, and βTG), logarithmic data transformation before analysis yielded normally distributed values. Antilog-transformed data are reported in this manuscript as mean and 95% confidence intervals of the back-transformed values. Linear regression analysis was used to examine the relationship between heparin plasma- and dimer-concentrations and F_{1+2} prothrombin fragments at the end of CPB. The chi-square analysis was used for categorical data. A P value of less than 0.05 was considered to be statistically significant.

Results

The data of all patients included in this study underwent subsequent analysis. Ten patients in group C were pretreated with intravenous heparin and five patients with subcutaneous heparin, compared with eight and seven patients, respectively, in group A. The duration of preoperative heparin treatment was 24 (CI 0.95: 16–32) and 29 (19–40) days for groups A and C, respectively (P = NS). Indication for heparin treatment was unstable angina and the risk of myocardial ischemia. Eight patients in group C and seven patients in group A were given antiplatelet therapy until the day before operation. The ejection fraction was less than 50% in five patients in group A and in three patients in group C. Mean body weight (77.3 [73–82] and 74.1 [68–80] kg for groups C and A, respectively) and age (62 [57–68] and 63 [59–67] yr for groups C and A, respectively) did not differ between the groups. The duration of operation was 270 (245–295) *versus* 229 (216–243) min in groups C and A, respectively ($P <$ 0.05). Although the CPB time and the aorta cross-clamp time was not different between the groups, the time of chest closure was significantly shorter in group A (49 [45–54] min) *versus* group C (67 [55–78] min; $P <$ 0.05; table 1).

Activated Clotting Time, Heparin Requirement, and Heparin Plasma Concentration

The lower of the two measured CACTs 10 min after heparin administration was 438 (397–501) and 588 (519–693) s for groups C and A, respectively ($P <$ 0.05) and was significantly prolonged during anticoagulation in group A compared with the control group; however, no differences were evident in the KACT (fig. 1). The mean CACT during the whole period of anticoagulation was 522 (471–581) and 760 (689–841) s for groups C and A, respectively ($P < 0.05$), and the mean KACT was 492 (435–556) and 518 (468–574) s for groups C and A, respectively (P = NS). In six patients—all of group C—one of the two CACTs did not reach 400 s after the initial bolus of 375 units/kg heparin. This was the indication for a repeat bolus of heparin. However, in all patients of the aprotinin group, the CACT substantially exceeded the target of 400 s. Ten minutes after the first heparin bolus, the KACT was 400 s or less in six patients in group A and seven patients in group C (fig. 2; P = NS). In seven patients in each group, the KACT was below 400 s at the end of CPB.

Total heparin administered was 36,200 (31,400–41,000) *versus* 27,700 (25,500–29,800) units for groups C and A, respectively ($P < 0.05$). Heparin plasma concentration at the end of CPB was significantly different: 3.2 (2.9–3.5) *versus* 2.6 (2.4–2.8) units/ml for groups C and A, respectively ($P < 0.05$) (fig. 3). Ten patients (all group C, but none in group A) received more than one heparin bolus. This additional heparin was given when one CACT did not reach 400 s after the initial bolus or when it decreased to less than this value during CPB.

Aprotinin Plasma Concentration

After 30 min on CPB, the plasma concentration of aprotinin was 181 (163–200) KIU/ml in group A (range 137 to 249 KIU/ml). At the end of operation, it was 127 (104–150) KIU/ml (range 77 to 210 KIU/ml).

Hemostatic Parameters

The courses of the F_{1+2} prothrombin fragments, thrombin-antithrombin III complex, and fibrin monomers are shown in figure 4. After 60 min of CPB all parameters were significantly ($P < 0.05$) different between the groups (group C vs group A): F_{1+2} prothrombin fragments, 9.7 (8.9–11.7) *versus* 7.5 (6.2–8.6) ng/ml; TAT, 53 (42–68) *versus* 29 (23–38) ng/ml; and fibrin monomers, 23 (12–43) *versus* 8 (3–17) ng/ml. D-dimer concentration at the end of operation was 656 (396–1,089) and 2,710 (1,811–4,055) ng/ml in groups A and C, respectively

Table 1. Demographic Data

Patient No.	Age (yr)	Weight (kg)	An
Control			
2	67	72	
4	48	90	
6	43	74	
7	57	70	
8	64	75	
12	67	58	
16	69	83	
17	72	79	
18	72	70	
19	48	85	
20	76	87	
23	69	79	
25	60	85	
28	65	75	
29	54	78	
Mean	62	77	
CI 95%	57–68	73–82	
Aprotinin			
1	65	83	
3	69	59	
5	57	71	
9	71	65	
10	63	69	
11	55	84	
13	63	74	
14	53	82	
15	62	56	
21	71	79	
22	77	75	
24	57	92	
26	61	62	
27	57	85	
30	63	75	
Mean	63	74	
CI 95%	59–67	68–80	

IMA = internal mammary artery; OP = oper

* Number of anastomoses (distal and proxir

† $P < 0.05$ *versus* control.

($P < 0.05$). There was no sig
tween the D-dimer concentrat
or fibrinmonomers at the end
plasma level, either within t
gregate data (fig. 5).

AT III activity before operat
compared with a normal plas
73% (65–81%) in group A (P
was in a pathologic range of <
tients in the control group d
AT III activity, 7 needed addi

Table 1. Demographic Data

Patient No.	Age (yr)	Weight (kg)	Anastomoses*	IMA	OP Time (min)	CPB Time (min)	6 h Blood Loss (ml)	Allogeneic Blood (units)	Comments
Control									
2	67	72	7	No	240	105	930	7	Rethoracotomy
4	48	90	3	Yes	193	50	600	1	
6	43	74	4	Yes	220	68	400	2	
7	57	70	5	Yes	280	86	1,100	3	Rethoracotomy
8	64	75	7	Yes	300	85	1,750	10	
12	67	58	6	No	270	104	1,600	11	Died
16	69	83	7	Yes	255	95	850	1	
17	72	79	8	Yes	280	111	1,200	1	
18	72	70	6	Yes	260	92	1,150	4	
19	48	85	4	Yes	260	75	1,270	5	
20	76	87	7	No	400	165	110	17	Died
23	69	79	7	Yes	245	109	580	8	
25	60	85	6	Yes	290	114	1,850	10	Rethoracotomy
28	65	75	7	Yes	280	91	1,500	10	
29	54	78	7	Yes	278	109	700	8	
Mean	62	77	6.1		270	97	1,039	6.5	
CI 95%	57–68	73–82	5.3–6.8		245–295	83–112	758–1,321	3.9–9.1	
Aprotinin									
1	65	83	5	Yes	240	87	530	1	
3	69	59	2	No	200	115	200	2	
5	57	71	5	Yes	210	70	310	2	
9	71	65	4	No	175	64	100	2	
10	63	69	7	Yes	240	113	220	5	
11	55	84	8	Yes	255	109	600	0	
13	63	74	5	No	215	106	180	1	
14	53	82	3	Yes	220	62	300	2	
15	62	56	5	Yes	210	74	300	7	Rethoracotomy
21	71	79	5	Yes	230	72	300	0	
22	77	75	7	Yes	230	93	480	2	
24	57	92	5	Yes	260	77	280	2	
26	61	62	5	Yes	245	79	400	5	
27	57	85	5	Yes	270	95	750	0	
30	63	75	5	Yes	240	70	450	2	
Mean	63	74	5.1		229†	86	360†	2.2†	
CI 95%	59–67	68–80	4.2–5.9		216–243	76–96	263–457	1.1–3.3	

IMA = internal mammary artery; OP = operation; CPB = cardiopulmonary bypass; CI = confidence interval.

* Number of anastomoses (distal and proximal) per patient.

† $P < 0.05$ *versus* control.

($P < 0.05$). There was no significant correlation between the D-dimer concentration, the F_{1+2} fragments, or fibrinmonomers at the end of CPB and the heparin plasma level, either within the groups or in the aggregate data (fig. 5).

AT III activity before operation was 74% (69–79%) compared with a normal plasma pool in group C and 73% (65–81%) in group A (P = NS). In 24 patients, it was in a pathologic range of <80%. Out of the 12 patients in the control group demonstrating a reduced AT III activity, 7 needed additional heparin to reach or maintain a CACT of more than 400 s. In contrast, none of 12 patients with reduced preoperative AT III activity in the aprotinin group received additional heparin. The aPTT 6 h after operation was significantly prolonged in the aprotinin group compared with the control group (group C, 42 (28–55) s; group A, 67 (57–77) s; $P < 0.05$).

Platelets

There were no differences in either platelet count (preoperative 238 [202–275] and 233 [187–280] ×

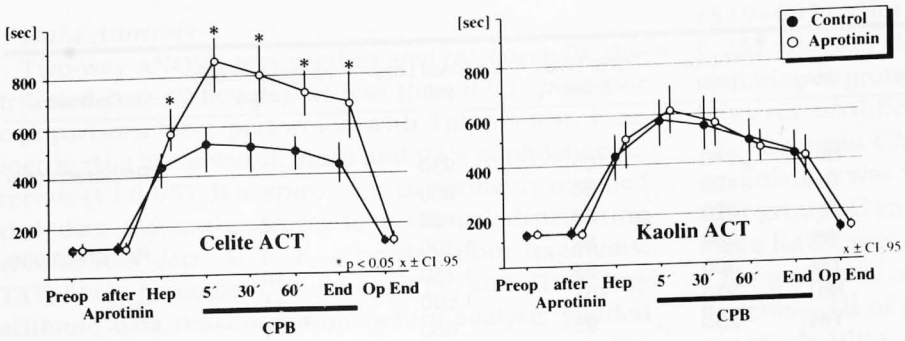

Fig. 1. Perioperative course of celite and kaolin ACT. Aprotinin causes a prolongation of the ACT when celite is used as an activator. There were no differences when kaolin was used. Data are given as mean; error bars indicate the 95% confidence intervals.

$10^5/mm^3$ in groups C and A, respectively) or in βTG and PF4 at any time point.

Blood Loss and Transfusion Requirements

Blood loss (fig. 6) was significantly different at all time points between the groups. At 24 h postoperatively, it was 1,496 (1,125–1,995) *versus* 597 (448–794) ml in groups C and A, respectively ($P < 0.05$). In group C, 6.5 (3.9–9.1) units of allogeneic blood were transfused perioperatively, compared with 2.2 (1.1–3.3) units in group A ($P < 0.05$). Four patients in group A were discharged from the hospital without transfusion, but all patients in the control group received allogeneic blood during the hospital stay ($P < 0.05$). There was no significant difference in the amount of red blood cells gained intraoperatively with the cell separator (696 [544–848] and 813 [603–1,022] ml in groups C and A, respectively). Only 3 patients in group A underwent postoperative shed mediastinal blood retransfusion (mean of the retransfused patients: 447 [238–654] ml); while in 12 patients, the drainage volume did not meet the criterion for retransfusion. In contrast, in group C, 12 patients received their drainage blood (mean amount in patients with retransfusion: 975 [739–1,211] ml; $P < 0.05$).

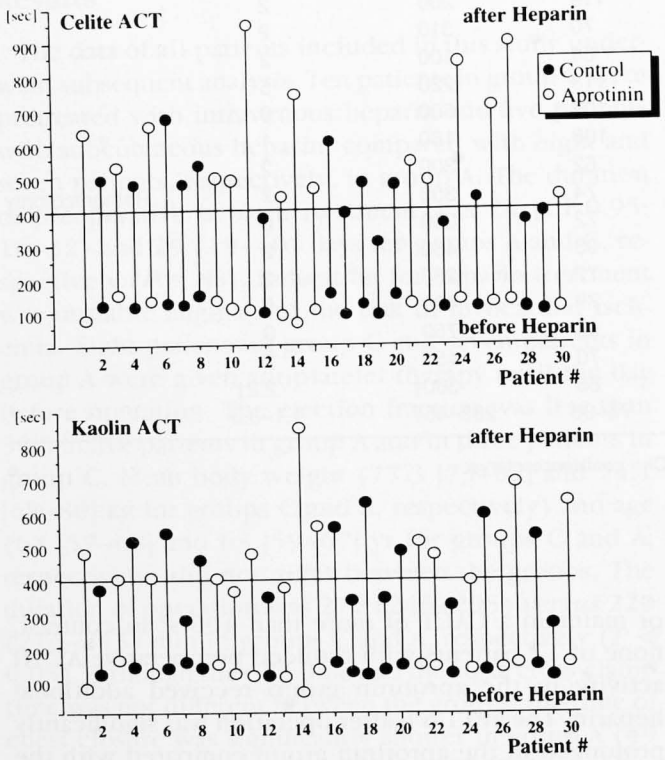

Fig. 2. Response of celite ACT and kaolin ACT to 375 units/kg heparin. The upper panel shows the individual CACT response to the first heparin bolus. Six of 30 patients, all of whom were members of the control group, failed to reach a CACT of 400 s or greater and were treated with a second bolus of 125 units of heparin. The lower panel represents the heparin response reflected by the kaolin ACT. When the KACT is used, there is no difference between the aprotinin and control group.

Hematocrit

The preoperative hematocrit was within normal ranges in both groups (group C, 41% [38–43%]; group A, 42% [39–46%]; P = NS). At discharge from the ICU, it was 33% (31–35%) and 36% (34–37%) in groups C and A, respectively ($P < 0.05$).

Outcome and Complications

One patient (group C) died of multiorgan failure during the first 30 postoperative days. Another patient (group C) died on postoperative day 41 of the complications of sepsis. Four patients (all group C) underwent rethoracotomy for surgical hemostasis. One patient (group A) was reoperated 6 h postoperatively because he exhibited signs of myocardial ischemia and circulatory instability. At the time of the second operation, all grafts were found patent and an additional vein graft was placed on the right coronary artery. In this patient, signs of myocardial ischemia (ST elevation

Fig. 3. Heparin plasma concent
Aprotinin-treated patients receiv
nificantly less heparin than did
patients. The heparin plasma con
tions at the end of CPB were signi
lower in the aprotinin group. Bef
eration and after protamine, no
heparin concentration could be d
Data are given as mean and 95% co
intervals.

and chest pain) were already
duction for his first operatio
creased from 1.3 (1.1–1.5) a
preoperatively to 1.5 (1.1–2.0
dl on postoperative day 7 for
tively, (P = NS). Four patien
group C showed an increase o
1 mg/dl within the first posto

Discussion

Aprotinin and Thrombin

Coagulation and fibrinolysis
even in the presence of cli
anticoagulation.[25] Because thr
in hemostatic activation du
thrombin generation and thro
degree of anticoagulation. In
tions (>200 KIU/ml), aproti
the contact phase of hemost
during CPB.[13] We hypothesi
dition to heparin, acts as an a
prothrombin activation durin
ticoagulant effect of aprotin
with heparin treatment before
are prone to develop reduce
sensitivity.

In the current study, F_{1+2}
thrombin-anti-thrombin III c
monomers were significantly
group. These results confirm
of aprotinin.[28] It is of note th
ulant activity was found wit
centrations that were often le

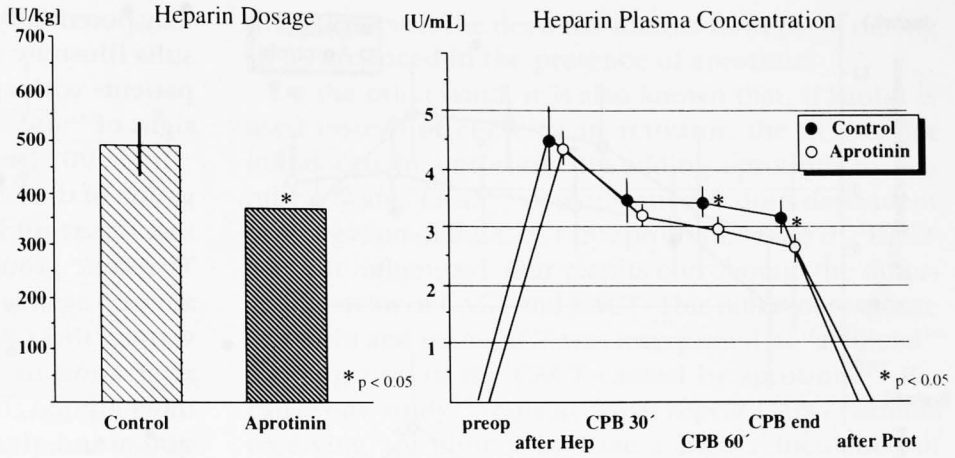

Fig. 3. Heparin plasma concentration. Aprotinin-treated patients received significantly less heparin than did control patients. The heparin plasma concentrations at the end of CPB were significantly lower in the aprotinin group. Before operation and after protamine, no residual heparin concentration could be detected. Data are given as mean and 95% confidence intervals.

and chest pain) were already evident before and at induction for his first operation. Plasma creatinine increased from 1.3 (1.1–1.5) and 1.2 (1.1–1.3) mg/dl preoperatively to 1.5 (1.1–2.0) and 1.8 (1.3–2.2) mg/dl on postoperative day 7 for groups C and A, respectively, (P = NS). Four patients in group A and one in group C showed an increase of creatinine of more than 1 mg/dl within the first postoperative week (P = NS).

Discussion

Aprotinin and Thrombin Generation

Coagulation and fibrinolysis are activated during CPB even in the presence of clinically sufficient heparin anticoagulation.[25] Because thrombin plays a central role in hemostatic activation during CPB, parameters of thrombin generation and thrombin activity indicate the degree of anticoagulation. In high plasma concentrations (>200 KIU/ml), aprotinin acts as an inhibitor of the contact phase of hemostasis[26,27] that is activated during CPB.[13] We hypothesized that aprotinin, in addition to heparin, acts as an anticoagulant by inhibiting prothrombin activation during CPB. To study this anticoagulant effect of aprotinin, we selected patients with heparin treatment before operation. These patients are prone to develop reduced intraoperative heparin sensitivity.

In the current study, F_{1+2} prothrombin fragments, thrombin-anti-thrombin III complex, as well as fibrin monomers were significantly reduced in the aprotinin group. These results confirm the anticoagulatory effect of aprotinin.[28] It is of note that this reduced procoagulant activity was found with aprotinin plasma concentrations that were often less than 200 KIU/ml. This finding indicates that aprotinin in plasma concentrations less than 200 KIU/ml may already attenuate contact phase activation. The improved anticoagulation in the aprotinin group was achieved despite a smaller heparin dosage and lower heparin plasma concentrations compared with the control group. The most likely explanation for this finding is that prothrombin activation takes place during CPB and is attenuated by aprotinin.[29]

Normal AT III activity is a prerequisite of heparin function.[30] The serine protease inhibitor AT III is the physiologic inhibitor of thrombin activity. Heparin catalyzes the speed of this reaction and increases the inhibitory activity of AT III dramatically. As a serine protease inhibitor,[31] aprotinin cannot enhance AT III function. Thus, Najman *et al.*[12] postulated an AT III independent anticoagulatory effect of aprotinin. Because of lowered AT III activity, patients under long-term anticoagulation with heparin develop reduced sensitivity to heparin during cardiac surgery.[19,20] Preoperative AT III activity was reduced in the current study in both groups. Compared with patients undergoing the same surgical procedure but without heparin pretreatment,[2] the need for a repeat bolus of heparin was increased in the control group.

Activated Clotting Time and Heparin Requirement

According to the protocol of this investigation, anticoagulation was monitored by the celite ACT. The CACT was more prolonged during the entire period of anticoagulation in the aprotinin group compared with group C. This is a well known effect of aprotinin.[14] The total amount of heparin used for anticoagulation during CPB was significantly lower in patients treated with

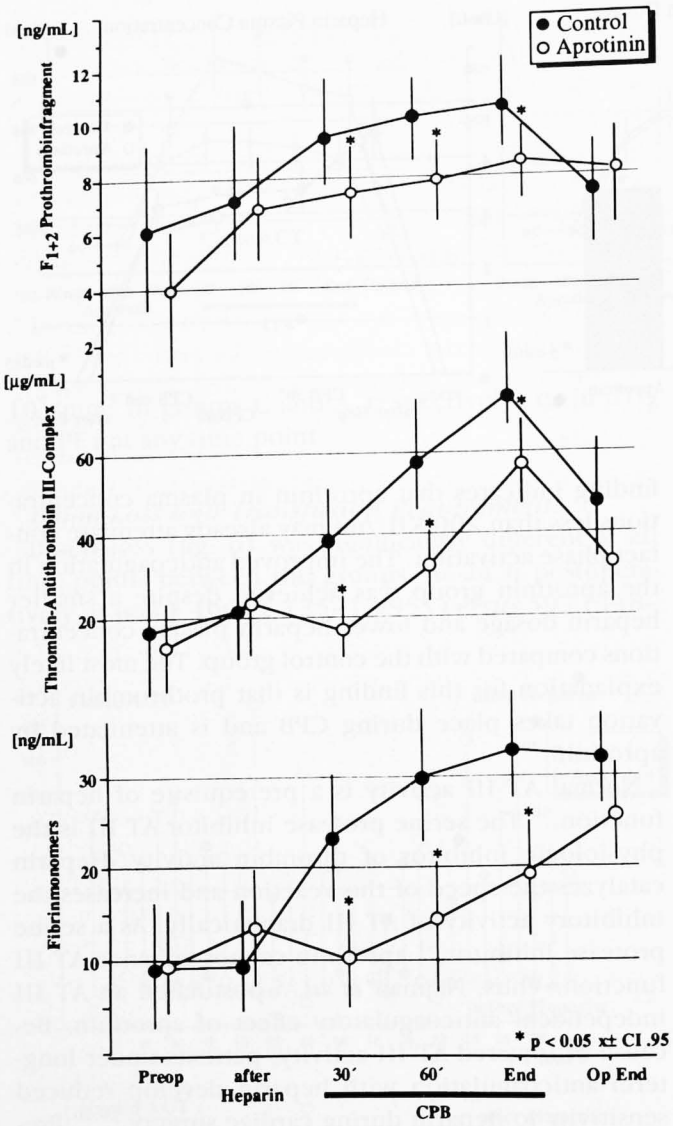

Fig. 4. Activation of coagulation. This figure shows the intraoperative course of indicators of thrombin generation and thrombin activity: F_{1+2} prothrombin fragments, thrombin-antithrombin-III complex, and fibrin monomers. The preoperative values were within the normal ranges of these test systems. In spite of heparin anticoagulation, thrombin was generated and fibrin was formed in both patient groups. However, activation of coagulation was significantly attenuated in aprotinin-treated patients. Data are given as mean and 95% confidence intervals.

aprotinin. This reduced dosage was reflected by a lower heparin plasma concentration at the end of CPB. The question is whether this reduction in heparin dosage reflects improved anticoagulation in the presence of aprotinin or whether the reduced heparin dosage is based on improper monitoring of anticoagulation and may potentially jeopardize patients' outcomes. Our results illustrate that hemostatic activation is reduced in patients with aprotinin treatment and that there are no signs of "underheparinization."[12]

In 1990, Desmet *et al.*[32] corroborated the prolongation of the celite ACT with aprotinin. They suggested that this would allow reduced heparinization for CPB. The same group, however, based on *in vitro* studies, advised against changing heparinization regimens and warned that CACT should not be used to monitor heparinization in aprotinin-treated patients.[33] These authors argued that aprotinin may inhibit the protein C system and, thus, further promote clotting through the extrinsic pathway. Reduced concentrations of F_{1+2}, TAT

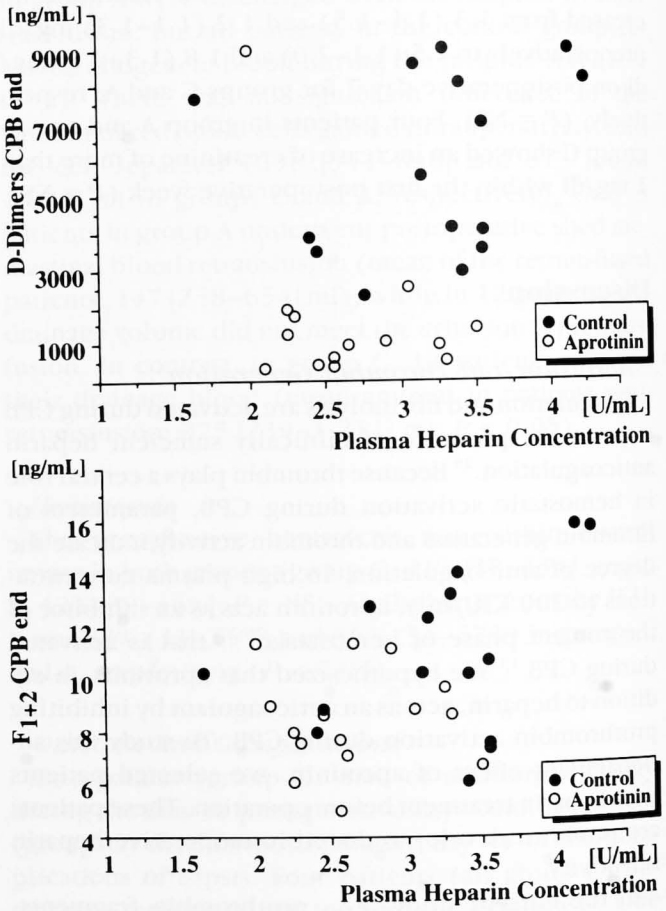

Fig. 5. Correlation of hemostatic activation and the heparin plasma concentration at the end of CPB. There was no significant correlation between heparin concentration and the activation of hemostasis, shown as D-dimer concentration (*top*) and F_{1+2} fragments (*bottom*), neither for all patients nor within the groups. Thus, measurement of heparin concentration did not give information about the degree of anticoagulation measured with these parameters.

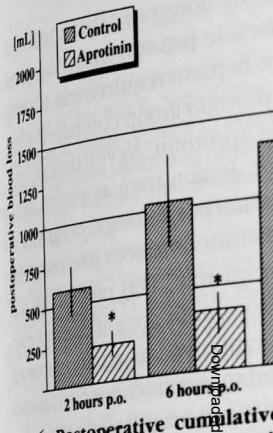

Fig. 6. Postoperative cumulative aprotinin-treated patients was si control patients. Data are given intervals.

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Some of the confusion in t of ACT in monitoring antico of aprotinin[34] is caused by "anticoagulation" and "hep is present, the CACT reflects duced anticoagulation, but th by both heparin and aprotini correlation between heparin even in the absence of apro correlation between heparin markers of activation of clott To estimate the degree of an evant than the measurement Because the measurement o trations does not reflect the h CPB, we must question the concentrations to monitor an

Does aprotinin have a hepa rent study demonstrates gre less heparin in the presence was not the aim of this stu heparin dosage for cardiac su phase activation is very impo is also the possibility of extr lation.[36] Only heparin is c pathway of activation. Becau patients in the current study ommend a reduced bolus o

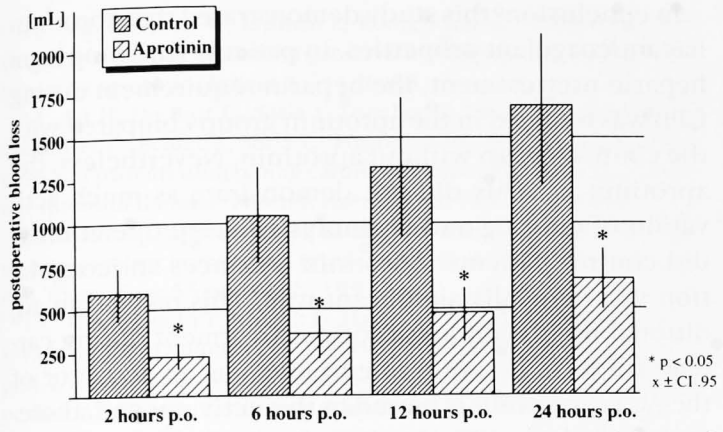

Fig. 6. Postoperative cumulative blood loss. Blood loss of aprotinin-treated patients was significantly less than that of control patients. Data are given as mean and 95% confidence intervals.

complex, and fibrin-monomers in the aprotinin group of the current study indicate that clotting activity was not increased. This finding would support the initial hypothesis of Desmet *et al.* that aprotinin acts as an anticoagulant and has a heparin-saving effect.[32]

Some of the confusion in the assessment of the role of ACT in monitoring anticoagulation in the presence of aprotinin[34] is caused by interchanging the terms "anticoagulation" and "heparinization." If aprotinin is present, the CACT reflects not only the heparin-induced anticoagulation, but the anticoagulation induced by both heparin and aprotinin. During CPB, there is no correlation between heparin plasma levels and ACT, even in the absence of aprotinin.[35] There is also no correlation between heparin plasma concentration and markers of activation of clotting or fibrinolysis (fig. 5). To estimate the degree of anticoagulation is more relevant than the measurement of heparin concentrations. Because the measurement of heparin plasma concentrations does not reflect the hemostatic changes during CPB, we must question the measurement of heparin concentrations to monitor anticoagulation.

Does aprotinin have a heparin-saving effect? The current study demonstrates greater anticoagulation with less heparin in the presence of aprotinin. However, it was not the aim of this study to find a safe minimal heparin dosage for cardiac surgery. The role of contact phase activation is very important during CPB, but there is also the possibility of extrinsic activation of coagulation.[36] Only heparin is capable of inhibiting this pathway of activation. Because of the small number of patients in the current study, it is not advisable to recommend a reduced bolus of heparin for anticoagulation. However, the need for additional heparin during CPB is reduced in the presence of aprotinin.

On the other hand, it is also known that, if kaolin is used instead of celite as an activator, the ACT is not influenced by aprotinin. By adding aprotinin to test tubes, Wang *et al.*[16] demonstrated a dose-dependent prolongation of the CACT by aprotinin, while the KACT was not influenced. Our results corroborate the different behavior of CACT and KACT. This different response of kaolin and celite ACT was interpreted as "artificial" prolongation of the CACT caused by aprotinin.[37] Because one study[38] indicated that repeat CABG patients receiving aprotinin may have a higher incidence of perioperative myocardial infarction—although the difference among the groups was not statistically significant—concern was raised regarding the method of monitoring intraoperative anticoagulation. Cosgrove *et al.*[38] speculated that the prolonged CACT with aprotinin tempts one to to lessen heparin therapy and, consequently, leads to inadequate anticoagulation. To avoid insufficient anticoagulation in the presence of aprotinin, it was suggested to discontinue use of the CACT,[12,19] to use a CACT of more than 750 s,[15] or to monitor heparin plasma concentration.[6]

We demonstrated[39] that the positively charged aprotinin is absorbed by the highly negatively charged kaolin, while it does not bind to celite. Thus, aprotinin is not able to inhibit contact activation *in vitro* in the presence of kaolin. The KACT reflects the anticoagulation by heparin alone and not the clinically relevant effects of aprotinin on coagulation. Therefore, the CACT is not "artificially" prolonged, but the KACT is "artificially" shortened. The KACT is recommended if the pure heparin effect on coagulation is of primary interest, *i.e.*, to detect residual heparin after protamine antagonization.

The aPTT was significantly prolonged in the aprotinin group, even 6 h after operation. This global test monitors coagulation from the activation of the contact phase of hemostasis to fibrin formation. Thus, because the contact phase activation is attenuated by aprotinin, the aPTT is useless for monitoring residual heparin after operation in the presence of aprotinin.

Bleeding and Allogeneic Blood Requirement

The clinical effectiveness of aprotinin in reducing intra- and postoperative bleeding in cardiac surgery has been well demonstrated in several studies.[2,3,6,8,31,40–42] In the current investigation, reduced activation of hemostasis as measured by reduced thrombin generation

in group A corresponded with a reduced intra- and postoperative bleeding tendency. Postoperative blood loss and blood requirement were significantly reduced by aprotinin. This reduction was in the same range as that found by Murkin *et al.*[42] in patients taking aspirin. Four patients in the control group had to be reoperated for surgical hemostasis. This was an unexpectedly large number of patients. Because this rate, as well as the amount of blood loss and blood requirement, are far from our daily routine,[3] it may reflect an increased risk of bleeding in patients with a preoperatively compromised hemostatic system.

One patient in the aprotinin group was reoperated because of unstable hemodynamic condition and signs of myocardial ischemia postoperatively. During reevaluation, an additional graft was placed on the right coronary artery. Electromagnetic flow measurement during reoperation revealed that all grafts were patent and no signs of graft occlusion were notable in this patient. Symptoms of impaired graft patency were not clinically evident in any of the aprotinin patients.

Limitations of the Study

First, the underlying presumption was that activation of coagulation measured by markers of thrombin generation plays the central role in hemostatic activation during CPB. Although there is strong evidence for this hypothesis, we cannot be sure that the parameters we determined are, in actuality, the key markers for hemostatic alterations. Additionally, there is no concise definition for "good" or "bad" anticoagulation. The term "underheparinization" still must be clearly defined. Although generally accepted, an ACT of less than 400 s as an indication for repeat heparin administration is an arbitrarily chosen cut point.

Second, to investigate patients with a strong likelihood of reduced heparin sensitivity, the entrance criterion for the study was heparin treatment for more than 10 days preoperatively. This is an uncommon patient population. Therefore, blood loss data do not reflect our routine experience with cardiac surgical patients.[2,3,14]

Third, only the intraoperative changes in hemostasis were evaluated. Because the postoperative period was not the focus of this study, it does not eliminate the possibility that aprotinin inhibits the early postoperative fibrinolysis driving hemostasis in a procoagulant direction during this time.

In conclusion, this study demonstrated that aprotinin has anticoagulant properties. In patients with long-term heparin pretreatment, the heparin requirement during CPB was reduced in the aprotinin group compared with the control group without aprotinin. Nevertheless, the aprotinin patients did not demonstrate as much activation of clotting and fibrinolysis during operation as did control patients. Aprotinin enhances anticoagulation in a clinically significant way. This may be an additional indication for aprotinin treatment during cardiac surgery. If kaolin is employed as an activator of the ACT, aprotinin is bound to this activator and, therefore, is unable to develop its anticoagulatory properties *in vitro*. In the current study, control of anticoagulation during CPB by the celite ACT produced reliable results, even in the presence of aprotinin.

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