
A Double-blind, Placebo-controlled Study

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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 (F1+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): F1+2 prothrombin fragments, 9.7 (8.9–11.7) ng/mL versus 7.5 (6.2–8.6) ng/mL; thrombin-antithrombin 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 CACT 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 (488–794) mL for groups C and A, respectively (P < 0.05).


The protease inhibitor aprotinin reduces blood loss after cardiac surgery. Activation of fibrinolysis is at...
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...tenuated by aprotinin treatment and platelet function is better preserved than in control patients after cardiopulmonary bypass (CPB). In addition, some evidence indicates that aprotinin also acts as an anticoagulant. 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. Aprotinin causes prolongation of celite-activated clotting time (CACT), 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. On the other hand, if kaolin is applied as an activator, the ACT (KACT) is not prolonged in the presence of aprotinin. Therefore, the KACT was recommended to control anticoagulation in patients treated with aprotinin.

Patients with heparin-induced anticoagulation for several days before operation are prone to develop reduced sensitivity to heparin. 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. 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 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 antithrombotic 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, 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 × 10^3 kallikrein inhibitor 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 (Hemocheck 800, Intern.

Cardiopulmonary Bypass

The extracorporeal circuit consisted of a Bernina separator (High Flex D 700, S, Innocor GmbH, Penne, Germany) and a nonocclusive roller pump system (Dadeco D 742, Mirandola, Italy). The oxygenator was primed with 12 l of Ringer solution, containing 5,000 units of heparin. Heparin-induced anticoagulation was performed in a state of hypothermia of 30–32°C at a flow rate of 2.4 l/min/m^2·m^-2. Myocardial ischemia was achieved by infusion of 1,000 ml of a bolus of crystalloid solution (Bretschneider, Chemie, Aschaffenburg, Germany) anticoagulated with 1,000 units of heparin. Myocardial ischemia was achieved by infusion of the bolus solution at the start of CPB and before reperfusion.

Transfusion Policy

The indication for allogeneic blood was based on the need for a hematocrit of 30% or less. Requirement for allogeneic blood was recorded during the week before surgery. A hematocrit of 30% was achieved with Ringer's lactate (Bretschneider, Chemie, Aschaffenburg, Germany) anticoagulated with 1,000 units of heparin. Myocardial ischemia was achieved by infusion of 1,000 ml of a bolus of crystalloid solution (Bretschneider, Chemie, Aschaffenburg, Germany) anticoagulated with 1,000 units of heparin. Myocardial ischemia was achieved by infusion of the bolus solution at the start of CPB and before reperfusion.

Digoxin (Lanoxin, Upjohn, Kalamazoo, MI) was used as a second-line agent for peripheral hypotension or tachycardia.

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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, 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 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), one roller pump, a cardiomyostomy 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/min 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. 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 (FDGP, Organon Teknika, Heidelberg, Germany). The complex of thrombin with antithrombin III (TAT), F₁₋₂, 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 (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.

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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 DITG), 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 ($P = N S$). 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 65 [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 A and C, 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 ($P = N S$). 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; $P = N S$). In seven patients in each group, the KACT was below 400 s at the end of CPB.

Total heparin administered was 56,200 (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 (25–38) 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 ($P < 0.05$). There was no significant difference between the D-dimer concentration or fibrin monomers at the end of operation or at any other time point during the operation. AT III activity before operation was 51% (65–81%) in group A ($P < 0.05$; fig. 4).}

Table 1. Demographic Data

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$Na =$ internal mammary artery; $OP =$ operation; $n =$ number of measurements (daily and proximal; $p =$ 0.05 versus control.)
Influence of High-Dose Aprotinin on Anticoagulation, Celite, and Kaolin Act

Table 1. Demographic Data

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Aprotinin

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CI 95% 59–67 68–80 4.2–5.9

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 F1+2 fragments, or fibrinogen levels at the end of CPB and the aprotinin 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 22 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 the 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 235 [187–280] ×

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10⁷/mm³ in groups C and A, respectively) or in βTG and PF-4 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 (1.1–3.5) 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 transfusion (mean of the transfused patients: 447 [238–654] ml); while in 12 patients, the drainage volume did not meet the criterion for transfusion. In contrast, in group C, 12 patients received their drainage blood (mean amount in patients with transfusion: 975 [739–1,211] ml; P < 0.05).

**Hematocrit**

The preoperative hematocrit was within normal ranges in both groups (group C, 41% [38–45%]; 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 and chest pain) were already detected for his first operation. One patient died from 1.3 (1.1–1.5) antiheparin antibodies preoperatively to 1.5 (1.2–2.0) antiheparin antibodies on postoperative day 7 for antiheparin antibodies (P = NS). Four patients in group C showed an increase of thrombin-antithrombin III complex (1 mg/dl) within the first postoperative day.

**Discussion**

**Aprotinin and Thrombin Antithrombin III Complex**

Coagulation and fibrinolysis were both normal in the presence of clinical anticoagulation.25 Because there was no difference in hematostatic activation due to thrombin generation and thrombin degree of anticoagulation. Increased (>200 IU/ml), aprotinin in the contact phase of hemostasis during CPB.13 We hypothesized that heparin acts as an antithrombin III activator during fibrinolytic effect of aprotinin with heparin treatment before aprotinin is prone to develop reduced sensitivity.

In the current study, F1.2-thrombin-antithrombin III complex concentrations were significantly different in group. These results confirm the beneficial effect of aprotinin.26 It is of note that aprotinin activity was found when heparin concentrations that were often low.
INFLUENCE OF HIGH-DOSE APROTININ ON ANTICOAGULATION, CELITE, AND KAOLIN ACT

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 (1.1–1.5) 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. 11 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, F1+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

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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.”

In 1999, Desmet et al. 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. 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 complex, and fibrin monomer in the aprotinin treated group and the current study indicate that aprotinin did not increase. This finding is consistent with the hypothesis of Desmet et al. that aprotinin acts as an anticoagulant and has a heparin-sparing effect.

Some of the confusion in the current study is caused by the clinician’s inability to distinguish between anticoagulation and “heparinization.” Some clinicians believe that “heparinization” and “anticoagulation” are synonymous. To estimate the degree of anticoagulation, we used a modified ACT assay (ACT.CAB). The modified ACT assay does not reflect the heparin concentration in the CPB circuit, and the concentration of heparin measured by the ACT.CAB assay is lower than the concentration of heparin measured by the ACT assay. To estimate the degree of anticoagulation, we used a modified ACT assay (ACT.CAB). The modified ACT assay does not reflect the heparin concentration in the CPB circuit, and the concentration of heparin measured by the ACT.CAB assay is lower than the concentration of heparin measured by the ACT assay.
INFLUENCE OF HIGH-DOSE APROTININ ON ANTICOAGULATION, CELITE, AND KAOLIN ACT

![Graph showing postoperative cumulative blood loss](image)

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.

The current study indicates 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 reaction 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 58 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. 59 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, 12,13 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 CACT 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 CACT 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. 2,3,5,8,31,40-42 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.,12 in patients taking aspirin. Four patients in the control group had to be operated 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,2 it may reflect an increased risk of bleeding in patients with a preoperatively compromised hemostatic system.

One patient in the aprotinin group was operated 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.2,3,11

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 anticoagulatory 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.

References

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