Influence of High-dose Aprotinin Treatment on Blood Loss and Coagulation Patterns in Patients Undergoing Myocardial Revascularization

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Intraoperative administration of the proteinase inhibitor aprotinin causes reduction in blood loss and homologous blood requirement in patients undergoing cardiac surgery. To ascertain the blood-saving effect of aprotinin and to obtain further information about the mode of action, 40 patients undergoing primary myocardial revascularization were randomly assigned to receive either aprotinin or placebo treatment. Aprotinin was given as a bolus of 2×10^6 kallikrein inactivator units (KIU) before surgery followed by a continuous infusion of 5×10^5 KIU/h during surgery. Additionally, 2×10^6 KIU were added to the pump prime. Strict criteria were used to obtain a homogeneous patient selection. Total blood loss was reduced from 1,431 ± 760 ml in the control group to 738 ± 411 ml in the aprotinin group ($P < 0.05$) and the homologous blood requirement from 838 ± 963 ml to 163 ± 308 ml ($P < 0.05$). In the control group, 2.3 ± 2.2 U of homologous blood or blood products were given, and in the aprotinin group, 0.63 ± 0.96 U were given ($P < 0.05$). Twenty-five percent of patients in the control group and 63% in the aprotinin group did not receive banked blood or homologous blood products. The activated clotting time as an indicator of inhibition of the contact phase of coagulation was significantly increased before heparinization in the aprotinin group (141 ± 13 s *vs.* 122 ± 25 s) and remained significantly increased until heparin was neutralized after cardiopulmonary bypass (CPB). The concentration of the thrombin–antithrombin III complex was significantly decreased at the end of CPB in the aprotinin group, indicating less thrombin generation in the aprotinin-treated group. The total concentration of the fibrinogen–fibrin split products (FSP) and the split products of the cross-linked fibrin (D-dimers) were also significantly reduced due to attenuated proteolytic activities of thrombin and plasmin. The results of the fibrin plate assay revealed higher fibrinolytic activity during CPB in the control group. The results demonstrate the beneficial effect of high-dose aprotinin treatment on blood loss and homologous blood requirement. This effect can be attributed to the inhibition of the contact phase of coagulation and the consequently reduced thrombotic and fibrinolytic activity during and after CPB. (Key words: Anesthesia: cardiac. Coagulation, cardiopulmonary bypass: aprotinin. Surgery: cardiac.)

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INTRAOPERATIVE ADMINISTRATION of the proteinase inhibitor aprotinin leads to a reduction in blood loss in cardiac surgery, thus decreasing the necessity for transfusion of banked blood.[1–6] The beneficial effect of other drugs, including desmopressin acetate (DDAVP, Rorer Pharmaceuticals, Ft. Washington, PA),[7] prostacyclin,[8] and dipyridamole,[9] on blood loss in patients undergoing cardiac surgery has already been proven in controlled clinical studies. Aprotinin is a naturally occurring enzyme inhibitor derived from bovine lungs. It acts on trypsin, plasmin, tissue-kallikrein, and to a lesser degree, on plasma-kallikrein.[10,11] Moreover, it is reported to have direct platelet-preserving properties in very high dosages.[12] Although aprotinin has been well known for many years and has been widely used for many surgical indications,[10] its blood saving properties have only become evident since it has been used in very high doses.[4] The rationale for these high dosages is to achieve aprotinin plasma concentrations during cardiopulmonary bypass (CPB) that are able to inhibit kallikrein activation.

Based on previous investigations,[5] we postulated that the clinical effect of aprotinin is based mainly on the inhibition of the contact phase of coagulation. During CPB, this system is activated by contact of blood with artificial surfaces of the extracorporeal circuit.[13] This activation cannot be completely inhibited by heparin. However, the postoperative bleeding tendency after cardiac surgery seems to be primarily due to impaired platelet function.[14] Intraoperative stimulation of coagulation with generation of thrombin may lead to platelet activation. It is therefore conceivable that additional inhibition of the contact phase of coagulation by aprotinin might cause a diminution of this bleeding tendency during and after CPB. However, the mechanism underlying the benefit from aprotinin has not yet been elucidated completely.

This prospective, double-blind, placebo-controlled

study was undertaken for two reasons: 1) to ascertain the blood-saving effect of high-dose aprotinin in a homogeneous group of patients undergoing cardiac surgery, and 2) to obtain further information about the mode of action of aprotinin.

Methods

Forty patients scheduled for elective primary myocardial revascularization gave informed consent to participate in this study, which had been approved by the local ethical committee. The study group comprised only male patients with preoperative normal left ventricular function (ejection fraction [EF] > 40%; left ventricular end-diastolic pressure [LVEDP] < 20 mmHg) and a preoperative hemoglobin concentration > 13.5 g/dl who were not receiving preoperative anticoagulant treatment. Antiplatelet therapy was stopped at least 5 days before surgery. Criteria for exclusion from the study were any surgical procedures other than revascularization, duration of operation greater than 360 min, and reexploration due to surgical bleeding, which was defined as an evident bleeding source found during reexploration.

Patients were randomly assigned to one of two groups: the aprotinin group (group A) and the control group (group C). Aprotinin and the respective placebo were provided by the manufacturer (Bayer AG, Leverkusen, FRG) in identical packages, each containing 12 bottles that could only be identified by the random number. Each bottle of aprotinin contained 5×10^5 kallikrein inactivator units (KIU) (= 70 mg) aprotinin in 50 ml 0.9% saline solution, and the placebo bottles contained only saline. The following dosage regimen was applied: after induction of anesthesia and before surgery, patients received a loading dose of 2×10^6 KIU aprotinin over a 15-min period followed by a continuous infusion of 5×10^5 KIU per h administered by an infusion pump for the entire duration of surgery. An additional bolus of 2×10^6 KIU was added to the pump prime of the heart–lung machine. Patients in group C received an equal volume of saline.

The indication for intra- and postoperative transfusion of homologous blood or blood products was defined as a hematocrit of less than 30%. The hematocrit was measured every 4 h within the first 24 h postoperatively and then every 8 h until discharge from the ICU.

Anesthetic, operative, and bypass management were standardized. Anesthesia was induced by flunitrazepam (0.02 mg/kg) and additional fentanyl (10–20 μg/kg). Pancuronium (0.1 mg/kg) was used to facilitate tracheal intubation. An arterial catheter *via* the radial artery and a pulmonary artery catheter *via* the right internal jugular vein were inserted after induction. Anesthesia was maintained with additional fentanyl. If necessary, enflurane or isoflurane was added during sternotomy.

Mucosa heparin (375 U/kg,Roche, Basel, Switzerland) was injected *via* a central venous catheter before aortic cannulation. Further heparin (125 U/kg) was administered if the activated clotting time (ACT) decreased below 400 s. The ACT was controlled every half hour during heparinization. The extracorporeal circuit consisted of a bubble oxygenator (Hiflex D 700, Dideco, Mirandola, Italy) that was primed with a 1,400 ml crystalloid solution containing 5,000 units of additional heparin, a cardiotomy reservoir (Dideco D 742, Mirandola, Italy), roller pumps, and polyvinyl tubing without an arterial filter. Cardiopulmonary bypass was performed with moderate hypothermia of 30° C 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 cardioplegic solution (Bretschneider HTK, F. Köhler Chemie, Alsbach, FRG, containing 9 mM potassium and 180 mM histidine) into the aortic root after aortic cross-clamping. After completion of CPB, residual heparin was neutralized with protamine chloride in a ratio of 1.5 mg per 125 U of the initial heparin dose. A cell separator without a special heparin administration suction line (Haemonetics, Munich, FRG) was only used to concentrate and wash the remaining blood of the oxygenator after termination of the bypass.

Mechanical ventilation was maintained postoperatively until peripheral rewarming and stable circulatory conditions were achieved and no major bleeding was noted. The reservoir of the heart–lung machine was used to collect shed mediastinal blood for retransfusion during the early postoperative phase. The drainage blood was retransfused up to 6 h after surgery if at least 250 ml were collected and its volume replacement was necessary.

Intra- and postoperative crystalloid and colloid infusion were recorded until the patient's discharge from the intensive care unit (ICU). Blood transfusions needed until discharge from the hospital were also recorded. Intraoperative blood loss was assessed by weighing the gauzes and sponges and measuring the content of the suction reservoir. The fluid used for rinsing was subtracted from this amount. The intraoperative bleeding tendency was assessed by the surgeon and the anesthesiologist after protamine administration using a score ranging from one (minimal) to four (excessive). Postoperative blood loss *via* the chest tubes was measured 6, 12, and 24 h postoperatively and at the removal of the chest tubes.

The red blood cell mass of the patients was calculated preoperatively, on the first postoperative day, and on discharge from the ICU by multiplying hematocrit with the blood volume,[15] which was derived from standard curves using the sex, age, and weight of the patient.

Blood samples were taken from the central venous catheter or the arterial port of the extracorporeal circuit at the following times: 1) after induction of anesthesia

before aprotinin infusion, 2) before heparin administration, 3) 5 min after the start of CPB, 4) 30 min after the start of CPB, 5) at the end of CPB, 6) after chest closure, 7) 2 h postoperatively, 8) on the first postoperative day, and 9) 7 days postoperatively. After discarding the first 10 ml, blood was drawn into EDTA tubes for platelet count, leukocyte count, and hematocrit or into acid-citrate-dextrose (ACD) solution (4:1) for all other measurements. The ACD blood was immediately 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 at −40° C in aliquots and thawed only before testing.

Tissue plasminogen activator (tPA) concentration, the split products of cross-linked fibrin (D-dimers; Boehringer Mannheim, FRG), total degradation products of fibrinogen and fibrin (FSP; Organon Teknika, Heidelberg, FRG), and the complex of thrombin with antithrombin III (TAT; Behring, Marburg, FRG)[16] were determined by sandwich ELISA using polyclonal and monoclonal antibodies. Calibration was performed with standard material supplied by the manufacturers. Results are given in nanograms per milliliter. Aprotinin plasma concentrations were quantified by means of a competitive ELISA according to Mueller-Esterl *et al.*[17] Total plasma protein was quantified with the Biuret method (Boehringer Mannheim, FRG). Spontaneous fibrinolytic activation in the native samples and in their (essentially inhibitor-free) euglobulin fraction was estimated on plasminogen containing human fibrin plates.[18] Any development of a lysis zone, regardless of its area, was considered as an indication of extrinsic plasminogen activator(s) in the sample.

To determine the ACT, 2 ml of blood were collected into vacuum tubes containing 12 mg celite. The Hemochron 800 (International Technidine Corp., NJ) was used according to the instructions of the manufacturer. Single measurements of ACT were performed every half hour and at all intraoperative measurement times. If the ACT exceeded 1,200 s, the measurement was stopped. Prothrombin time, thrombin time, and activated partial thromboplastin time (aPTT) were measured by routinely applied clotting methods. Bleeding time was determined after induction of anesthesia, after neutralizing the heparin effect with protamine chloride, and 4 h postoperatively in the ICU. Since the ear lobe is the only part of the body within the reach of the anesthesiologist during surgery, samples were taken from this body part. The subaqual bleeding method that is a modified Duke method was applied.[19]

Summary data of all variables are, if not otherwise stated, expressed as mean ± SD. Analysis of variance (ANOVA) was used if appropriate. The Mann-Whitney *U* test was applied where data did not follow a normal distribution according to the results of Shapiro's test for normality. The chi-squared test was used for categorical data. $P < 0.05$ was considered statistically significant.

Results

Data of 20 patients in group C and 19 patients in group A were evaluated. One patient (group A, patient 4) was excluded from analysis because of an additional intraoperative procedure (resection of a left ventricular aneurysm) that led to a prolongation of the operation. Reexploration due to surgical bleeding was not necessary.

As shown in table 1, patients in the two groups were comparable in terms of age, weight, operation, and CPB time. Thirteen patients in group C and 11 patients in group A also had additional internal mammary artery implantation.

Intraoperative blood loss per patient was 636 ± 322 ml in group C and 363 ± 159 ml in group A ($P < 0.05$). The postoperative blood loss is given in figure 1. The mean cumulated loss 6, 12, and 24 h postoperatively per patient was 721 ± 471 ml, 894 ± 491 ml, and 1,169 ± 605 ml, respectively, in group C and 303 ± 209 ml, 399 ± 251 ml, and 584 ± 295 ml, respectively, in group A. The total postoperative blood loss per patient until removal of the chest tubes was 1,431 ± 760 ml in group C and 738 ± 411 ml in group A ($P < 0.05$). One patient (patient 37) in group C had a total blood loss of 3,550 ml. Since all hemostatic parameters were within the normal range, this blood loss was probably due to surgical bleeding; however, reexploration was not performed in this patient. Even after excluding this patient from analysis, the difference in blood loss between the groups was still significant ($P < 0.05$) at each stage. A total of 663 ± 171 ml per patient (group C) and 731 ± 283 ml per patient (group A) autologous packed cells gained with the cell separator from the oxygenator were retransfused (not significant [NS]). Two patients in group C and one patient in group A received homologous blood intraoperatively. A mean of 431 ± 367 ml of drainage blood (maximum, 1670 ml [patient 37]) and 147 ± 188 ml of drainage blood (maximum, 810 ml [patient 39]) was retransfused *via* the reservoir postoperatively in group C and A, respectively. The mean amount of intra- and postoperatively transfused whole blood was 838 ± 963 ml per patient in group C and 163 ± 308 ml per patient in group A ($P < 0.05$). A total of 2.3 ± 2.2 U per patient of homologous blood or blood products (*i.e.*, blood, packed cells, or fresh-frozen plasma) were given in group C and 0.63 ± 0.96 U per patient in group A ($P < 0.05$). In group C, 46 U of homologous blood were transfused to 15 patients, representing a mean of 3.1 U per patient; whereas in group A, 12 U were given to seven patients, representing a mean

TABLE 1. Patient Data

Group C						Group A					
Patient No.	Age (yr)	Weight (kg)	IMA	Op. Time (min)	CPB Time (min)	Patient No.	Age (yr)	Weight (kg)	IMA	Op. Time (min)	CPB Time (min)
1	62	72	Yes	420	152	3	64	79	No	260	133
2	51	69	Yes	230	48	7	56	76	Yes	185	53
5	51	81	No	180	45	8	69	75	Yes	225	117
6	56	74	No	210	61	9	64	77	No	175	70
10	51	87	Yes	220	91	13	67	87	No	220	83
11	63	79	Yes	250	83	14	61	76	Yes	250	85
12	65	68	No	155	39	15	49	81	Yes	210	73
16	57	62	Yes	165	33	18	49	81	No	200	73
17	58	74	No	205	60	19	73	69	No	175	55
21	54	75	Yes	260	75	20	57	77	No	239	111
22	58	82	No	125	25	23	67	77	Yes	240	77
24	68	63	Yes	220	79	26	67	93	No	315	130
25	49	72	Yes	205	80	27	48	66	Yes	225	75
30	47	90	No	180	57	28	47	87	Yes	230	82
31	46	99	Yes	315	93	29	61	66	Yes	205	63
32	58	76	Yes	235	53	34	37	67	Yes	265	91
33	38	63	Yes	230	91	35	68	98	No	295	116
37	45	85	Yes	320	120	36	48	84	Yes	195	60
38	64	67	Yes	300	93	39	57	78	Yes	335	133
40	56	73	No	170	56						
Mean ± SD	55 ± 8	76 ± 10		230 ± 68	72 ± 31		58 ± 10	79 ± 9		234 ± 45	88 ± 27

IMA = internal mammary graft.

There were no significant differences of the variables between the groups.

of 1.7 U per patient. Twenty-five percent of the patients in group C and 63% in group A were discharged without receiving any banked blood or homologous blood products.

Intraoperative blood loss correlated significantly with time of operation in group C, whereas it did not in group A (fig. 2). The correlation between total blood requirement and duration of operation was also only significant ($P < 0.05$) in group C.

The bleeding score as an assessment of intraoperative bleeding tendency (ranging from minimal [1] to excessive

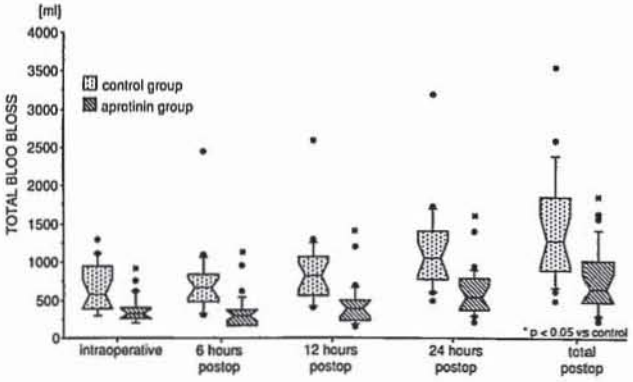

FIG. 1. Box plot of intra- and postoperative blood loss. The top of the box represents the 75th percentile, the line in the middle the median, the bottom the 25th percentile, and the notches the 95% confidence bounds. The top and the bottom of the whiskers are associated with the 90th and 10th percentile, respectively, and the circles represent values above and below these percentiles. The postoperative data are cumulative values. The topmost circle in the control group represents a patient (patient 37), who had a total postoperative blood loss of 3,550 ml. Even after removing this patient from analysis, however, the difference between the groups still remained significant.

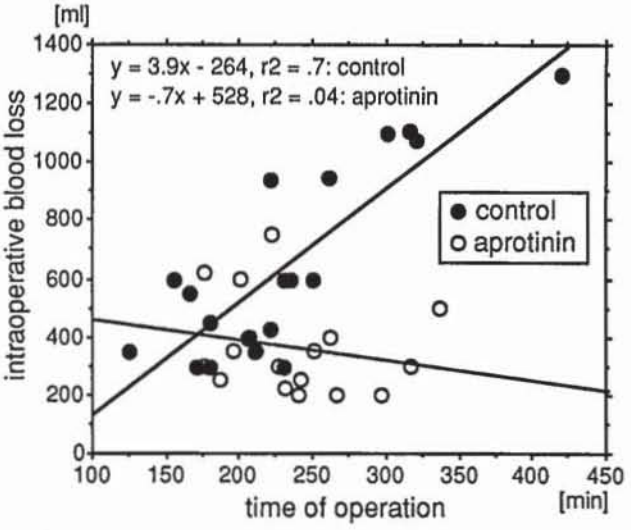

FIG. 2. Correlation between intraoperative blood loss and duration of operation. The intraoperative blood loss was assessed by weighing the gauzes and sponges and measuring the content of the suction reservoir. Blood loss and time of operation correlated significantly in the control group but did not correlate in the aprotinin group.

[4]) was 3.15 in group C and 2.3 in group A ($P < 0.05$). The hematocrit on the first postoperative day was 33.7 ± 4.1% in group C and 37.9 ± 2.9% in group A ($P < 0.05$). The preoperatively calculated red blood cell mass was 2,428 ± 283 ml and 2,572 ± 300 ml in groups C and A, respectively (NS). At discharge from the ICU, it differed significantly: 1,846 ± 234 ml in group C and 2,027 ± 168 ml in group A ($P < 0.05$). This difference remained significant until the seventh postoperative day when the red blood cell mass was 1,954 ± 312 ml in group C and 2,137 ± 214 ml in group A ($P < 0.05$). The elapsed time between end of CPB and chest closure was 55 ± 19 min in group C and 46 ± 11 min in group A (NS). After CPB, the preoperative platelet count dropped from 213 ± 58 × 10^9/l to 129 ± 58 × 10^9/l in group C and from 193 ± 44 × 10^9/l to 115 ± 47 × 10^9/l in group A (NS).

The ACT, which was in a comparable range preoperatively in the two groups, was significantly increased 5 min before administration of heparin in group A (141 ± 13 s *vs.* 122 ± 25 s) and remained significantly higher until antagonizing the effect of heparin after CPB. The aPTT was also significantly increased before heparin in group A (34 ± 2.8 s *vs.* 74 ± 7.3 s; $P < 0.05$) and remained significantly prolonged until 2 h after surgery (66 ± 23 s *vs.* 45 ± 25 s). The preoperative bleeding time in groups C (180 ± 45 s) and A (178 ± 27 s) were within the normal range; however, 4 h postoperatively, it was 213 ± 46 s in group A and 290 ± 114 s in group C ($P < 0.05$).

There were no differences in the total plasma protein concentrations between the groups throughout the study period. The concentration of the TAT complex is given in figure 3. The differences between the groups 30 min after start of CPB and at the end of CPB were significant ($P < 0.05$) with 48 ± 21 ng/ml and 82 ± 42 ng/ml in group C compared to 24 ± 11 ng/ml and 42 ± 14 ng/ml in group A.

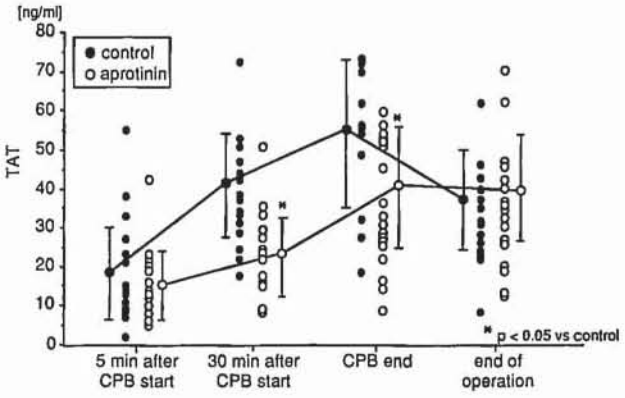

FIG. 3. Course of the thrombin–antithrombin III complex (TAT) during extracorporeal circulation and at the end of operation. Each dot or circle represents a single value, whereas the lines connect the mean ± SD values.

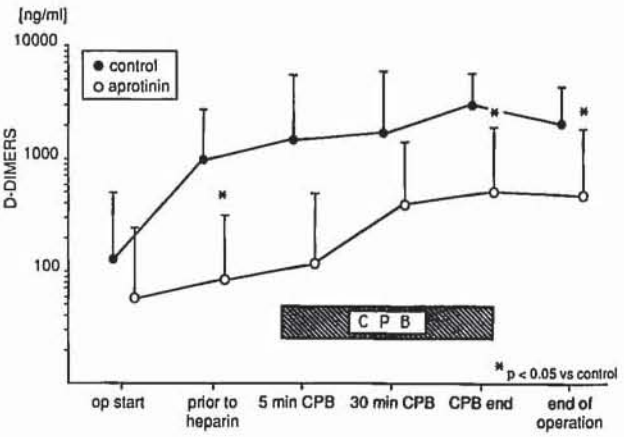

FIG. 4. Concentration of the split products of cross-linked fibrin (D-dimers). All values are mean ± SD. During CPB, there was a significantly increased generation of D-dimers in the control group as compared to the aprotinin group, indicating less fibrin formation and less fibrinolytic activity in the aprotinin-treated patients.

The concentration of the split products of the cross-linked fibrin (D-dimers) increased in both groups during surgery (fig. 4). The increase was less in the aprotinin group, however, and was 532 ± 1,425 ng/ml and 497 ± 1,398 ng/ml 30 min after onset of CPB and at the end of CPB, respectively, which is significantly less than the values of group C (2,155 ± 2,300 ng/ml and 3,131 ± 2,755 ng/ml). The FSP concentrations showed a similar course (fig. 5). At the end of CPB, the concentration was 10,824 ± 7,261 ng/ml in group C and 2,510 ± 3,932 ng/ml in the group A ($P < 0.05$).

The fibrin plates revealed increased fibrinolytic activity during the entire course of CPB in the control group

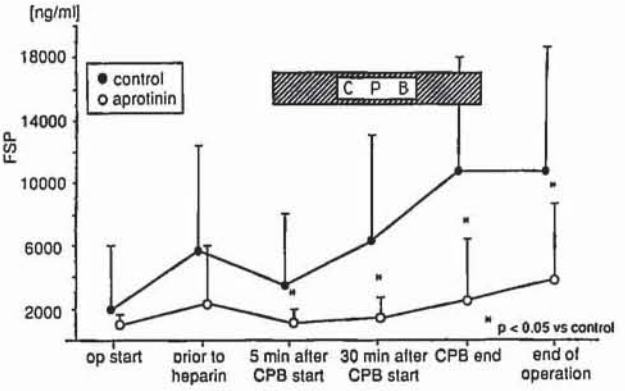

FIG. 5. Total degradation products of fibrinogen and fibrin. Values shown in the graph are mean ± SD. During the whole period of CPB, there was a significant increase in the control group as compared to the aprotinin group. However, comparison of the preoperative value and the concentration at the end of CPB reveals a significant increase in the aprotinin group as well.

TABLE 2. Fibrinolytic Activity on Fibrin Plates

Time	Preparation	Control Group (mm^2)	Aprotinin Group (mm^2)	P
Prior to operation	Euglobulin	29 ± 11	23 ± 11	NS
	Native	0	0	
Prior to heparin	Euglobulin	36 ± 20	17 ± 15	<0.05
	Native	0	0	
5 min after start of CPB	Euglobulin	48 ± 20	15 ± 15	<0.05
	Native	6 ± 9	0	<0.05
30 min after start of CPB	Euglobulin	72 ± 25	41 ± 28	<0.05
	Native	23 ± 21	3 ± 4	<0.05
End of CPB	Euglobulin	77 ± 33	68 ± 30	NS
	Native	33 ± 27	7 ± 6	<0.05

The values represent the area of the lysis spots on the fibrin plates. The area is proportionate to the fibrinolytic activity in the plasma. The euglobulin row represents the values after removal of the inhibitors of fibrinolysis by the euglobulin preparation. All values are given as mean ± SD.

(table 2). In the native samples, fibrinolytic activity was evident in 12 patients 5 min after onset of CPB and in 13 patients 30 min after onset of CPB in the control group; however, this could not be demonstrated 5 min after the start of CPB and in eight patients 30 min after the start of CPB in the aprotinin group ($P < 0.05$). At the end of CPB, however, fibrinolytic activity could be seen in 15 patients in group C and in 14 patients in group A. The quantitative measurement of the lysis spots is given in table 2. At the end of the operation, no differences could be determined. In contrast, the tPA concentration was not significantly different during the course of surgery. It increased from 6 ± 3.7 ng/ml preoperatively to 18 ± 10 ng/ml at the end of CPB in group C and from 6 ± 2.3 ng/ml to 16 ± 9 ng/ml in group A. None of the laboratory results showed any significant difference after the first postoperative day.

The aprotinin plasma concentration (fig. 6) demonstrated an increase from 152 ± 61 KIU/ml before heparin to 335 ± 106 KIU/ml 5 min after onset of CPB. Thereafter, a continuous decrease was found until the end of CPB (191 ± 62 KIU/ml). Two hours after surgery, the aprotinin concentration was 74 ± 31 KIU/ml.

The postoperative course of all patients was uneventful. No clinically relevant side effects could be attributed to aprotinin treatment.

Discussion

This study clearly demonstrates the influence of high-dose aprotinin treatment given during the entire course of open heart surgery on intra- and postoperative blood loss. Comparing homogeneous patient groups, a highly significant reduction of intra- and postoperative blood loss was found in the aprotinin-treated group. This reduction led to a concomitant saving of homologous blood. Postoperative blood loss was reduced by 48%, whereas the banked blood requirement was diminished by nearly 80%. The reduced bleeding tendency was clinically evident as shown by the different bleeding scores given for the two groups by the surgeon and the anesthesiologist. This study corroborates the results of our previous investigation[5] and those of others[2,4] that also show a numerical discrepancy between the degrees of postoperative bleeding reduction and transfusion requirement. This discrepancy may be explained by the fact that, due to intraoperatively decreased blood loss, the red blood cell mass or hematocrit remains higher as an effect of aprotinin treatment, thus lessening the need for homologous blood transfusion. Furthermore, the hemoglobin content and, consequently, hemoglobin loss *via* chest tubes are diminished in this patient group, where the drainage fluid consists mainly of inflammatory exudation.[4]

The differences between the two groups with regard to bleeding and banked blood requirement agree with

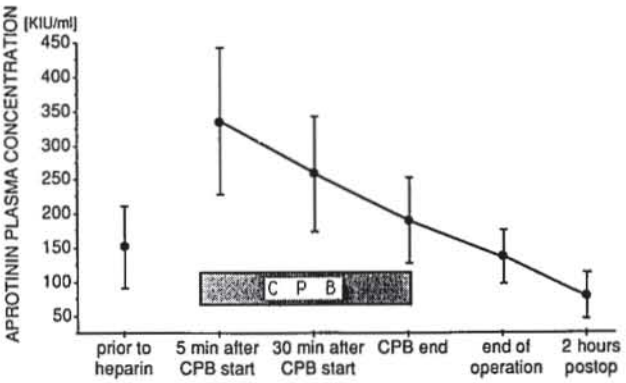

FIG. 6. Aprotinin plasma concentration. The peak value 5 min after the onset of CPB represents the bolus of 2×10^6 KIU aprotinin given to the pump prime. Despite the continuous infusion of 5×10^5 KIU aprotinin per h the concentration decreased towards the end of CPB and towards the end of operation. Values are given as mean ± SD.

the results of other studies using the same or a similar aprotinin dosage regimen. All studies using aprotinin during coronary artery bypass grafting showed nearly identical reductions in blood loss: Bidstrup *et al.*, 46%[2]; Van Oeveren *et al.*, 47%[20]; and Fraedrich *et al.*, 46%[3]. However, the reduction in a group of patients with miscellaneous types of operations was 29%.[5] The blood-saving effect in these studies varied between 43[5] and 88%.[4] This blood-saving effect is due not only to reduction of postoperative blood loss but also to diminished intraoperative bleeding tendency as shown in the current study.

The strong correlation between the duration of CPB and intraoperative blood loss, which was evident in the patients of the control group, could not be demonstrated in the aprotinin-treated patients.

Nevertheless, the mode of action of aprotinin is not yet completely clear. Capillary bleeding and oozing in cardiac surgery are believed to be due to impaired platelet function. The most important consequence of CPB is the loss of platelet aggregability.[13,21] Platelet activation occurs as a direct result of the contact of blood with a synthetic surface during CPB. The influence of aprotinin on platelet adhesive receptors could be demonstrated in one study.[20] Consequently, a direct platelet-preserving property of aprotinin has been postulated.[20]

The effect of aprotinin on platelets—of primary or secondary nature—is unquestionable. In accordance with others,[2] we observed a shorter postoperative bleeding time with aprotinin treatment as a result of better preserved platelet function; however, the platelet count did not differ between both groups during the whole operation.

The surface-mediated activation of the contact system of coagulation involves the interaction of factor XII (Hageman factor) and kallikrein (besides high molecular weight kininogen and factor XI).[22] Aprotinin may inhibit kallikrein. Without the amplifying effect of kallikrein on the conversion of factor XII to XIIa, the contact phase activation is inhibited or takes place only slowly. Major consequences of the surface-mediated activation are the stimulation of both the intrinsic pathway of coagulation[23] with the effect of thrombin formation and the propagation of the fibrinolytic pathway leading to plasminogen activation. While activation of the contact system has no major direct effect on platelet activation,[24] thrombin is a powerful platelet activator. Therefore, it is conceivable that the effect of aprotinin on platelets is secondary to the inhibition of the contact system of coagulation.

Indeed, the significant reduction of the D-dimers and the thrombin–antithrombin III complex concentration found in the current study indicates less thrombin generation, and the results achieved with the fibrin plates demonstrate less fibrinolytic activity during aprotinin application. The inhibition of the contact phase of coagulation due to aprotinin is responsible for this reduced thrombin formation. Both diminished thrombin generation and fibrinolysis caused less formation of the fragments of fibrinogen and fibrin.

Aprotinin is a powerful plasmin inhibitor.[25] While we observed differences in the results of the fibrin plates between the groups, we could not detect any significant difference in the course of tPA concentration. The immunoassay, however, may not differentiate free and complexed tPA. Plasma kallikrein inhibition not only leads to reduced contact activation of coagulation but can also cause reduced fibrinolytic activation.[24,26] The current findings indicate that endothelial activation of fibrinolysis is not inhibited by aprotinin, whereas the activation *via* plasma kallikrein is attenuated. The course of the ACT, which is an indicator of contact activation,[27] and the aPTT elevation with aprotinin treatment refer to the inhibition of the contact phase of coagulation. These results strongly suggest that the inhibition of the contact phase of coagulation is the primary effect of aprotinin action and therefore responsible for the blood-saving effect of this drug. This finding is in contrast to the interpretation given by van Oeveren *et al.*[20] who focused on the direct platelet protective effect of aprotinin on the specific platelet receptors. Inhibition of fibrinolysis and the better preserved platelet function are of secondary nature.

The rationale for choosing the given aprotinin dose regimen was to get a constant aprotinin plasma concentration of more than 200 KIU/ml, which is supposed to be the threshold of plasma kallikrein inhibition.[28] The time course of plasma aprotinin concentration revealed that the aprotinin dose used did not maintain a stable concentration throughout CPB. However, the inhibitor concentration exceeded 200 KIU/ml after onset of CPB. It might be possible that a higher aprotinin concentration at the start of CPB is desirable because surface activation takes place mainly during the initial phase of CPB. The course of the fibrin–fibrinogen split products and the results of the fibrin plates demonstrated that aprotinin, at least in the dosage given, was not able to completely suppress the ongoing hemostatic activation. All parameters showed an increase toward the end of CPB. Further investigation is needed to ascertain whether this tendency can be prevented with another (*i.e.*, higher) dose regimen of aprotinin during CPB. The interindividual differences in the aprotinin levels do seem to warrant the application of a body weight-oriented dosage of aprotinin instead of a fixed dose.

We conclude that high-dose aprotinin treatment has a highly beneficial effect on the hemostatic mechanism during and after CPB, leading to a substantial reduction of intra- and postoperative bleeding tendency. The most likely mechanism of aprotinin action is the inhibition of kallikrein. The current results show that the dosage of

aprotinin does not provide a constant aprotinin concentration during CPB. Further studies are required to delineate the precise mode of aprotinin action and to determine the most effective aprotinin dosage.

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