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The effect of two different doses of aprotinin on hemostasis in cardiopulmonary bypass surgery: similar transfusion requirements and blood loss

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ABSTRACT

Background and Objectives. **Various dosages of aprotinin have proven to be effective in reducing blood loss and allogeneic transfusion requirements in cardiopulmonary bypass surgery, despite the controversy surrounding the precise hemostatic mechanisms of this drug. The aim of our prospective, randomized, double-blind study was to assess differences in blood loss and transfusion requirements and the effect of two dosages of aprotinin on hemostatic activation.**

Design and Methods. **Patients undergoing coronary artery bypass grafting received high-dose aprotinin (n=28), pump-prime-only (PPO) aprotinin (n=28), or placebo (n=28).**

Results. **The high-dose and the PPO groups had a significantly lower blood loss (985 mL [95%CI 845-1,102] and 1,255 mL, [95% CI 1,025-1,406], respectively) than the placebo group (1,416 mL, 95%CI 1,248-1,612]. Transfusion requirements were lower in the aprotinin-treated groups than in the patients receiving placebo (21 units and 15 units in the high and low-dose groups vs 59 units in placebo group). As far as concerns parameters of thrombin generation, the aprotinin groups showed a significant reduction of F1+2 prothrombin fragment but not of thrombin-antithrombin complexes. There were higher levels of natural anticoagulants, i.e. antithrombin, protein C and protein S, in the high-dose aprotinin group. As regards fibrinolysis parameters, D-dimer was lower in the aprotinin groups, and the levels of α_2-antiplasmin and plasmin-α_2-antiplasmin complexes were raised. In summary, both dosages of aprotinin were equally effective in reducing blood loss and transfusion requirements. There was a lower activation of coagulation and fibrinolysis in cardiopulmonary bypass patients treated with aprotinin: the levels of natural anticoagulants were less decreased in the high-dose group. No differences in thrombotic complications were observed between aprotinin groups.**

Interpretation and Conclusions. **Our study shows that both dosages of aprotinin are safe and effective in reducing transfusion requirements. Considering the difference in cost of using a low-dose or high-dose schedule, the former should be recommended for patients undergoing cardiopulmonary bypass surgery.**

Key words: cardiopulmonary bypass; blood loss; aprotinin; hemostasis; transfusion requirements.

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Given its proven efficacy in reducing the tendency to bleed and allogeneic blood requirements, high-dose aprotinin was recently introduced as a standard treatment during cardiac operations in adults and children undergoing cardiopulmonary bypass (CPB) surgery.[1-3] Aprotinin was first described for clinical use in cardiovascular operations in 1964.[4] Most studies are based on the so-called Hammersmith regimen,[1] a high-dose aprotinin schedule, the efficacy and safety of which has been widely demonstrated.[4-13] Aprotinin is a non-specific proteinase inhibitor from bovine lung, belonging to the kunins superfamily.[14] It inactivates human serine proteinases such as trypsin, plasmin, kallikrein, and activated protein C, in a dose-dependent way, although the precise mechanism is under discussion.[4,6,8,15] The fibrinolytic components of the hemostatic system are activated during CPB surgery. There is an increase in fibrinogen degradation products and plasminogen activators with a concomitant decrease in plasminogen and α_2-antiplasmin in the patient's plasma and a shortening of the clot lysis time. However, it is still unclear whether this enhanced fibrinolysis is a major contributor to post-surgical bleeding in a significant number of patients.[4] Other authors have considered the role of platelet dysfunction and the inflammatory mediators of the complement system and the cytokine network. Controversial results have been reported concerning the possible protective mechanism of action of aprotinin on platelets, although some authors suggested that the hemostatic effect of aprotinin is not mediated by protection of platelet function.[16-19] Nevertheless, the hemo-

static mechanism of aprotinin seems to be related to its inhibitory effect on the activation of the clotting system, and on the inhibition of hyperfibrinolysis.[3,5,8,15,18]

Some authors have studied the possibility of introducing other schedules with lower doses of aprotinin, taking into account the economic repercussions and the reduction of the potential secondary adverse effects of aprotinin.[2,9,10,20-22] Only few reports have published data on different dosages of aprotinin and on the consequences on blood requirements and hemostatic activation during CPB surgery.[3,7,9,21–24]

The aim of this study was to determine the effects of two dosage schedules of aprotinin in order to evaluate the drug's dose-related efficacy in terms of blood loss and transfusion requirements, whilst clarifying its contribution to the improvement of the acquired hemostatic defect associated with CPB.

Design and Methods

Patients

A prospective, randomized, double blind study was performed on 84 patients (75 males and 9 females) undergoing coronary artery bypass grafting. All patients were advised of the procedures and their associated risks in accordance with institutional guidelines and gave informed consent. In order to be eligible for the study, the patient had to be older than 18 years. The following exclusion criteria for entry to the study were used: history of previous cardiac operation, possible exposure to aprotinin in the past, allergy or clotting disorder, severe cardiac failure (ejection fraction <30%), and impaired renal function (serum creatinine level >2 mg/dL). Demographic data are summarized in Table 1.

Aprotinin administration and cardiopulmonary bypass technique

Aprotinin (Trasylol,® Bayer aG, Leverkusen, Germany) was administered in a saline solution without additives or preservatives. The patients were randomized into three groups depending on the dose of aprotinin or placebo. *Group H*: the high-dose group received a bolus of 2×10^6 KIU of aprotinin, 2×10^6 KIU of aprotinin in the priming solution and a continuous infusion of aprotinin (5×10^6 KIU hourly) during CPB (n=28). *Group L*: the low-dose (pump-prime-only) group received a bolus of saline, 2×10^6 KIU aprotinin in the priming solution and a continuous infusion of saline during CPB (n= 28). *Group P*: the placebo group received a bolus of saline. Saline was also added to the priming solution and a continuous infusion of saline was administered during CPB (n=28).

Table 1. Demographic data and data from CPB.

Characteristic	*Low-dose*	*High-dose*	*Placebo*	*p value*
Nº patients	28	28	28	
Age (yr)	61 (40-75)	58 (38-78)	59 (41-76)	NS
Sex (m/f)	24/4	27/1	24/4	NS
Duration of CPB (min, mean ± SD)	117±34	103±30	116±36	NS

Anesthesia and CPB

Anesthetic, surgical and CPB procedures were carried out according to the protocols of the Hospital de Sant Pau (Barcelona, Spain) and were similar in all three groups. Anesthesia was induced with flunitrazepam (0.02 mg/kg body weight) and fentanyl (10 to 20 µg/kg body weight). Pancuronium bromide (0.1 mg/kg body weight) was used for muscle relaxation. Anesthesia was maintained with successive doses of fentanyl. Isoflurane (0.5-1%) was added to the ventilation system when needed. The CPB device was primed with Ringer's solution, polygeline, and mannitol. Extracorporeal circulation was performed at 28ºC with an output of 2.2 to 2.4 L/m^2 per minute. The perfusion pressure was maintained between 50 and 80 mmHg. A cardioplegic solution containing mannitol (8.9 g/L), dextrose (4.5 g/L), potassium (30 mmol/L), chloride (113 mmol/L), sodium (82 mmol/L), and bicarbonate (20 mmol/L) was injected every 20 minutes into the aortic root.

Anticoagulation was achieved with heparin (300 IU/kg body weight) injected into the right atrium. Successive doses of heparin were given during CPB. Given that aprotinin prolongs the activated clotting time (Hemochron 401 device, International Technidyne Corp., Edison, NJ, USA), patients in whom the first activated clotting time measurement exceeded 750 seconds received heparin in similar doses to maintain the activated clotting time between 750 and 950 seconds. The activated clotting time was maintained between 400 and 500 seconds in the patients in whom the first activated clotting time measurement was about 400 seconds. All groups received heparin in similar dosages. When surgical procedures were completed and the CPB was discontinued, heparin neutralization with protamine sulfate was achieved by means of an infusion of 1.5 mg for each 100 IU of the heparin administered.

Blood sampling

Blood samples were taken immediately before the operation (T_1), at the end of CPB but before protamine administration (T_2), 10 minutes after protamine administration (T_3), 1 hour after protamine neutralization (T_4) and 18-24 hours after the operation (T_5). Blood mixed with 0.129 mmol/L sodium citrate in a 10:1 proportion was obtained for analysis. The samples were centrifuged within 30 min at 3,600 rpm at 4ºC. Aliquots of the plasma were snap-frozen and stored at –40ºC until analysis.

Laboratory assessments

Hemoglobin and platelet counts in whole blood were measured by routine analysis with a Coulter counter (Coulter Electronics, Luton, England). Other parameters measured were: D-dimer (normal range: <500 µg/L; Asserachrom D-dimer, Boehringer-Mannheim, Mannheim, Germany), prothrombin fragment F_{1+2} (normal range: 0.36-1.4 nmol/L; Enzygnost F_{1+2} micro, Behring, Marburg, Germany). Plasma tissue factor was analyzed by ELISA (normal range: 63-126 ng/L, Immubind Tissue Factor, American Diagnostica, USA), and functional tissue factor pathway inhibitor was assayed as described elsewhere (normal range: 55-150%).[25] Protein C antigen (normal range: 70-130%; Asserachrom (Protein C, Boehringer-Mannheim), total protein S antigen (normal range: 73-124%; Asserachrom total protein S, Boehringer-Mannheim, Germany) plasmin-α_2-antiplasmin complexes (normal range: 80-470 mg/L; Enzygnost PAP micro, Behring) were measured by ELISA. The analyses of α_2-antiplasmin antigen (normal range: 80-110%) were performed by immunodiffusion. Antigenic measurements rather than functional ones were employed to avoid laboratory artifacts given the presence of aprotinin in the blood samples.

Blood loss measurements and transfusion requirements

Intra-operative blood loss was estimated by weighing the gauze sponges and measuring the contents of the reservoir of the suction device. The fluid used for rinsing was subtracted from this amount. The volume of blood loss drained from the chest tubes was measured intra-operatively and cumulative blood loss at 6, 12 and 24 hours after CPB. The transfusion criteria were as follows: red blood cells were administered when the hemoglobin concentration was less than 80 g/L (70 g/L in the CPB period) or when the patient was in shock because of hemorrhage. Fresh frozen plasma was given if microvascular bleeding was present and the prothrombin ratio exceeded 1.5 or the fibrinogen level was less than 1 g/L. Platelet concentrates were given in cases of microvascular bleeding and when the platelet count was less than 50×10^9/L.

Statistical analysis

The results are expressed as means and standard deviations, or as 95% confidence intervals (CI). Percentages are expressed with their CI. All relevant differences were shown as the CI of these differences. A *p* value of less than 0.05 was considered statistically significant. Age, sex, and duration of operation and extracorporeal circulation were compared between groups by one-way analysis of variance. Primary efficacy analysis was performed on the total blood loss during the first 24 hours after the operation by an analysis of variance with two factors (treatment and CPB duration). An analysis of variance for repeated measures with one factor (treatment) was carried out to analyze the blood profiles at different times, and CPB duration was used as a covariable. Polynomial contrasts were applied within different trends of each treatment. Bonferroni's correction was applied for multiple comparisons. Multiple regression analysis was performed to evaluate the relationship between overall blood loss and some clinical and biological variables. Differences between percentages of patients receiving transfusions were estimated with the χ^2 method. In patients receiving transfusions, differences in the number of blood units were assessed by analysis of variance with CPB duration as a covariable. A logistic regression model was used to estimate the odds ratio of undergoing transfusion in each treatment group. Age, sex, CPB duration, pre-operative hemoglobin concentration, and pre-operative platelet count were included as adjustment variables. The improvement in –2 log likelihood was evaluated to establish the goodness-of-fit of the model. The evolution of biological parameters throughout the CPB period was assessed by analysis of variances for repeated measures (treatment as the factor and duration of CPB as the covariable). Polynomial contrasts were applied to evaluate profiles between treatments. If no influence of the analyzed factors was observed, a simple analysis of variance for repeated measures was used. D-dimer, PAP, and prothrombin fragment values were analyzed with the use of logarithmic transformation.

Results

Eighty-four patients were randomized. No significant differences were noted in age, sex, and CPB time between the three groups. Intra-operative blood loss in females was greater than in males ($p<0.001$). The duration of CPB influenced intra-operative blood loss in all groups ($p<0.001$). Data are summarized in Table 2.

Table 2. Blood loss and transfusions requirements.

	Low-dose	*High-dose*	*Placebo*
Blood loss (mL)			
Intra-operative	626 (523-793)	498 (416-580)	644 (532-756)
6 hr post-operative	332 (249-414)*	222 (171-272)°	422 (249-414)
12 hr post-operative	445 (340-550)*	310 (248-373)°	571 (454-686)
24 hr post-operative	629 (504-755)*	487 (391-583)°	772 (647-896)
Total blood loss	1255 (1025-1406)*	985 (846-1101)°	1416 (1248-1612)
Post-operative need of packed red cell units (% patients)			
0 Units	89%	89%	61%
2 Units	7%	11%	32%
4 Units	4%	0	7%
Total units	21 U	15U	59U

*Blood loss values given as mean and 95% CI. *p<0.005 placebo group versus low dose group; °p<0.0005 placebo group versus high dose group.*

Table 3. Biological parameters related to thrombin generation.

	Low dose (n=28)	*High dose (n=28)*	*Placebo (n=28)*
Prothrombin fragment (nmol/L)			
T1	1.6 (1.4-1.9)	1.9 (1.5-2.2)	2.0 (1.5-2.6)
T2	7.1 (4.7-9.6)°	5.1 (3.6-6.7)*	12.0 (6.1-17.9)
T3	6.5 (4.4-8.6)°	5.0 (3.3-6.7)*	13.5 (5.5-21.5)
T4	6.9 (5.1-8.7)°	6.7 (4.9-8.4)*	11.2 (5.6-16.8)
T5	1.8 (1.4-2.2)	2.3 (1.7-2.9)	1.9 (1.6-2.4)
Total protein S (%)			
T1	94.0 (84.3-103.7)	99.2 (91.1-107.2)	94.4 (87.7-101.2)
T2	60.0 (52.3-67.3)	72.4 (62.3-82.5)	58.6 (52.3-64.9)
T3	62.0 (54.5-68.6)	69.4 (61.1-77.7)	59.6 (53.0-66.1)
T4	66.2 (58.6-73.8)	75.4 (65.8-84.9)*	60.5 (50.6-70.4)
T5	63.5 (55.9-71.1)°	72.0 (60.7-83.3)*	50.7 (45.9-55.4)
Protein C antigen (%)			
T1	95.9 (87.9-103.9)	98.2 (89.1-107.4)	94.1 (87.5-100.6)
T2	52.1 (51.9-60.1)#	59.4 (54.2-64.7)*	51.9 (46.7-57.1)
T3	56.0 (51.9-60.0)	62.6 (56.8-68.4)*	52.1 (46.5-57.7)
T4	58.5 (54.0-63.1)#	67.4 (61.5-73.4a	54.7 (49.4-59.9)
T5	57.3 (52.8-61.7)#	66.6 (60.0-73.2)*	51.9 (47.4-56.5)
Tissue factor pathway inhibitor (%)			
T1	164.3 (124.9-203.6)	136.5 (106.2-166.9)	152.6 (107.3-197.9)
T2	292.1 (190.6-393.6)	275.5 (205.5-345.6)	251.6 (199.3-303.9)
T3	139.3 (93.5-185.1)	134.7 (108.3-161.2)	196 (137.7-201.4)
T4	137.8 (99.6-176.0)	151.2 (116.9-185.4)	142.5 (115.4-169.5)
T5	93.5 (63.4-123.5)	101.6 (81.7-121.6)	89.8 (61.7-117.8)
Tissue factor (ng/L)			
T1	90.3 (52.7-127.9)	110.9 (86.0-135.8)	116.9 (92.1-141.8)
T2	42.7 (26.2-59.2)	67.8 (44.7-90.9)	61.8 (45.1-78.6)
T3	44.7 (26.4-63.0)	74.5 (49.8-99.3)	65.9 (50.2-81.6)
T4	49.0 (27.6-70.4)	77.3 (57.9-96.6)	72.8 (55.6-90.1)
T5	74.9 (62.2-87.6)	74.9 (62.2-87.6)	73.5 (54.4-92.6)

*T_1, before operation; T_2, before protamine; T_3, 10 min after protamine; T_4, 1 hour after protamine and T_5, 18-24 hours after CPB. °p<0.05 between low-dose and placebo group. #p<0.05 between low and high-dose groups. *p<0.005 between high-dose and placebo group.*

There were no significant differences in intra-operative blood loss between the three groups. The volume of post-operative blood loss was significantly lower in the high and low-dose aprotinin groups than in the placebo group ($p<0.05$ and $p<0.005$ respectively). No statistical differences were found in post-operative blood loss between the low and high-dose groups. The total blood loss (intra-operative plus 24 hour-post-operative loss) was significantly less in the low (1,255 mL; 95% CI: 1,025-1,406) and high-dose (985 mL; 95% CI: 845-1,101) groups compared with in the placebo group (1,416 mL, 95% CI: 1,248-1,612). There was a linear correlation between overall blood loss and levels of D-dimer in the placebo group, but this correlation did not exist in the low-dose (regression coefficient 0.07, 95% CI: 0.01-0.51) or high-dose group (regression coefficient 0.2, 95% CI: 0.03-0.96). D-dimer did not change significantly during CPB in the aprotinin group, and hence, no correlation with blood loss was found. In the placebo group, higher levels of D-dimer correlated with greater blood loss.

Transfusion requirements

Eighty-nine per cent of the patients in the low-dose and high-dose groups did not receive blood components; 61% of the patients in the placebo group did. Data are summarized in Table 2. There were no significant differences between the low and the high-dose groups. The reduction in the number of packed red cells given after the operation was statistically different in the high-dose group from that in the placebo group ($p<0.02$). The transfusion requirements were lower in the low-dose group compared with in the placebo group. Although the differences were not significant 6 and 12 hours after the operation ($p=0.06$), by 18-24 hours, the low dose group received fewer blood products ($p<0.05$).

Adverse events

Adverse events in the high-dose group consisted of one case of hypertension, one complete atrio-ventricular block, two cerebrovascular accidents and one case of myocardial infarction. All of theses resolved within 24 hours, and none of them seemed to be related to the aprotinin use. In the low-dose group (pump-prime-only dose), one case of myocardial infarction was recorded. Finally, two cases of myocardial infarction were diagnosed in the placebo group.

Biological parameters

Parameters related to thrombin generation

The results are summarized in Table 3. Plasma tissue factor (TF) levels decreased during the operation, but ten minutes after protamine neutralization the levels were similar to the pre-oper-

Table 4. Biological parameters related to plasmin activity.

	Low-dose (n=28)	*High-dose (n=28)*	*Placebo (n=28)*
D-dimer (µg/L)			
T_1	425 (343-506)	561 (400-723)	600 (454-745)
T_2	619 (474-765)°#	433 (323-543)*	1836 (1347-2326)
T_3	1059 (266-1853)°	500 (369-631)*	1798 (1335-2262)
T_4	660 (508-812)°	540 (396-684)*	2192 (827-3556)
T_5	515 (420-609)°	582 (463-702)*	884 (731-1037)
Plasmin-α2-antiplasmin complexes (mg/L)			
T_1	460 (401-520)	446 (391-501)	475 (424-526)
T_2	540 (438-642)°#	931 (588-1275)	1194 (780-1608)
T_3	996 (437-1556)°	1113 (741-1486)	1323 (923-1722)
T_4	712 (598-826)°#	958 (648-1267)	1021 (862-1181)
T_5	154 (96-212)#	227 (185-269)	158 (114-203)
α2-antiplasmin (%)			
T_1	97.4 (92.9-101.9)	98.3 (92.9-103.6)	94.1 (88.9-99.5)
T_2	48.3 (43.9-52.7)#	56.8 (52.7-60.8)*	46.1 (42.4-49.9)
T_3	51.8 (47.1-56.5)	58.1 (53.9-62.3)*	46.8 (42.5-51.1)
T_4	56.7 (51.7-61.7)°#	64.5 (60.2-68.7)*	49.1 (44.4-53.8)
T_5	75.2 (70.0-80.39)	79.2 (72.9-85.5)*	68.6 (62.5-74.6)

*T_1, before operation; T_2, before protamine; T_3, 10 min after protamine; T_4, 1 hour after protamine and T_5, 18-24 hours after CPB. °p<0.05 between low-dose and placebo group. #p<0.05 between low and high-dose groups. *p<0.005 between high-dose and placebo group.*

ative ones. Tissue factor pathway inhibitor (TFPI) levels increased during CPB in all groups but after 24 hours the levels returned to those recorded pre-operatively. As for F_{1+2} prothrombin fragment (F_{1+2}) levels, a marked increase was observed after CPB. In the placebo group, F_{1+2} levels were significantly higher than in the groups treated with low and high-dose aprotinin ($p<0.05$ and $p<0.005$, respectively). No statistical differences were observed between the low and high-dose groups. The total protein S antigen (PS) and protein C (PC) antigen levels decreased during CPB in all groups and rose slightly from the time of protamine neutralization to 24 hours after CPB. The PS levels in the high and low-dose aprotinin groups were significantly higher one hour after protamine and 24 hours after CPB than those in the placebo group ($p<0.005$ and $p<0.05$, respectively). No differences were observed between the low and high-dose groups. In the high-dose group, PC levels were significantly less reduced than in the low-dose and placebo groups. This difference persisted at 24 hours ($p<0.05$ and $p<0.005$, respectively). In summary, the extent of coagulation activation was smaller in the groups treated with aprotinin, although a slightly more reduced consumption of natural anticoagulants was observed in the high-dose group.

Plasminogen activation and plasmin activity parameters

The results are summarized in Table 4. The course of D-dimer levels in the placebo group differed significantly from that in the low and high-dose groups ($p<0.05$ and $p<0.005$, respectively). D-dimer levels increased sharply during and after CPB in the placebo group whereas the increment was very low in the high and low-dose aprotinin groups. Only during CPB were there statistical differences between the low and high-dose groups ($p<0.05$), the D-dimer levels being higher in the low-dose group. The α_2-antiplasmin antigen levels decreased in all the groups. The decrease was less marked in the high-dose group. Although the levels in the low-dose group were slightly higher than those in the placebo group, the differences were significant only after CPB. The plasmin-α_2-antiplasmin complexes increased during CPB in all the groups. The levels were lower in the low-dose group than in the other groups. Briefly, a more intense suppression of fibrinolysis activation was observed in the high-dose group. Nevertheless, in the low-dose group the levels of the plasmin-α_2-antiplasmin complexes were lower: this could be attributed to a combined effect of low plasmin generation and lower levels of α_2-antiplasmin.

Discussion

A number of studies have demonstrated the clinical effectiveness of a high dose of aprotinin in improving hemostasis in patients undergoing cardiopulmonary bypass surgery, and consequently in reducing bleeding.[1-3,8-10,15] Nevertheless, only few authors have reported the efficacy of lower doses of aprotinin, including minimal,[20] half[26] or pump-prime-only dosages.[17,20] In all these studies, a reduction in blood loss and transfusion requirements was observed. Seven prospective, randomized, double-blind studies comparing different aprotinin dosage schedules in adults have been published[3,7,9,21-24] and 2 in children.[2,3] Only four of them compared a pump-prime-only dose of aprotinin with other dosages.[7,9,21,22] In terms of efficacy, the results obtained in theses studies demonstrated that a high, low or pump-prime-only dose of aprotinin reduces blood loss and the need for transfusions. Only Levy *et al.* did not find a superior efficacy of pump-prime-only doses compared with placebo in patients undergoing repeated procedures.[9] In our prospective, double-blind randomized study, aprotinin at high or low-doses was more effective than placebo. Our findings are consistent with those of other authors.[7,21,22] Low-dose and high-dose aprotinin reduced bleeding and the need for allogeneic blood units: this effect was more marked when the

higher dose was employed (21 units versus 15 units respectively), but did not reach statistical significance. Both doses, therefore, seem to have a similar effect in reducing transfusion requirements. The question of the safety of aprotinin, in relation to its dose, also arouses controversy. Lemmer *et al.* reported an increased risk of cardiovascular complications. Based on Royston's hypothesis, these authors argued that high-dose aprotinin inhibited both coagulation and fibrinolysis, but that at lower doses it inhibited only fibrinolysis without effectively inhibiting coagulation. This could result in a hypercoagulable state, which could give rise to thrombotic complications.[7] In our study, we found no differences in adverse effects between the groups, which is consistent with the findings of other authors.[9,21,22]

In the literature, only two trials in adult patients have tried to shed light on the mechanisms of different aprotinin dosages by measuring biological parameters related to coagulation and fibrinolysis.[6,22]

Dietrich *et al.* concluded that high doses were significantly more effective than lower doses, (although a pump-prime-only dose was not included in that study) in attenuating fibrinolysis.[6] Speekenbrink *et al*. also reported complete fibrinolysis inhibition as a result of high doses and only partial attenuation following a pump-prime-only dose although thrombin generation was lower in both groups.[22] In our study, the effects of a low-dose (pump-prime-only) and a high-dose of aprotinin on hemostatic activation during cardiopulmonary bypass surgery did not differ significantly. However, the natural inhibitor system was more preserved in the high-dose group. These data suggest that the extent of coagulation activation is lower when aprotinin is used, especially in the case of higher doses. Thus, these findings are in agreement with the view that aprotinin, at high doses, acts as an anticoagulant and an antifibrinolytic, but at low-doses, the inhibition of coagulation may be less that that of fibrinolysis. Despite these biological findings, in practice, thrombotic events did not differ between groups. The size of our sample was too small for side effects to be assessed and indeed the study was not designed for that purpose; therefore, it is not possible to rule out a higher rate of thrombotic complications in any group.

It has been suggested that tissue factor is overexpressed in monocytes during extracorporeal circulation.[27,28] We measured plasma tissue factor but we did not observe differences during cardiopulmonary bypass. There was a decrease in all groups, that could have been related to hemodilution. Blood-borne tissue factor is now considered to play a key role in thrombus progression.[27] Aprotinin has structural similarities with tissue factor pathway inhibitor (TFPI), and a possible TFPI-like effect of aprotinin is likely.[4] We measured functional TFPI levels but no differences between the groups were observed. TFPI levels increased during the extracorporeal circulation in the heparinization period. Heparin is negatively charged and leads to a release of TFPI from the endothelium-bound pool. This inhibitor has been recently cloned and has a strong homology to basic protease inhibitors of the Kunitz-type such as aprotinin. It has been postulated that aprotinin, in combination with the well-known effect of heparin during CPB, may boost the effect of TFPI as well as inhibit its degradation.[4] Other authors have studied its mechanisms, which seem to be related to a protective effect of fibrin-rich thrombi already generated without influencing platelet-rich thrombus formation.[29,30] Our findings do not exclude a possible TFPI-like effect of aprotinin given that the increase in TFPI due to heparin may mask the TFPI-like effect of aprotinin. A direct inhibition of coagulation activation triggered by tissue factor due to aprotinin warrants further investigation.

As for fibrinolysis system measurements, changes in parameters related to plasminogen activation and plasmin activity demonstrate an inhibitory effect of aprotinin on enhanced hyperfibrinolysis induced by cardiopulmonary bypass. This reflects an attenuation of fibrinolysis with a smaller production of plasmin-generated fibrin degradation products, and a lower consumption of plasminogen and α_2-antiplasmin. Although the levels of plasminogen and α_2-antiplasmin did not differ statistically in the low and high dose groups, plasmin- α_2-antiplasmin complexes were lower in the low-dose group. Plasmin inhibition and the protection of hemostatic plugs could constitute important mechanisms, reducing bleeding in patients undergoing cardiopulmonary bypass surgery.

In summary, we found a dose-dependent attenuation of the deleterious effect of CPB on hemostatic activation by aprotinin. The administration of high or low-doses of aprotinin led to lower coagulation activation, a less marked decrease in natural inhibitors, and had an inhibitory effect on the enhanced fibrinolysis after CPB. Higher doses of aprotinin showed a dose-related higher activity in hemostatic activation after CPB, acting as an anticoagulant and an antifibrinolytic agent: we did not observed fewer thrombotic complications in this group. In our study, these differences did not significantly affect the need for allogeneic blood products. Both doses were effective in reducing transfu-

sion requirements and no important adverse effects were observed. Taking into account the economic benefit and simplicity of the low-dose aprotinin schedule, the pump-prime-only schedule may be acceptably effective in reducing blood loss in patients undergoing cardiopulmonary bypass surgery.

Contributions and Acknowledgments

AS designed the study, was responsible for the data management, and prepared the manuscript. JM also designed the study and supervised the study. AO was responsible for data analysis. JM collected all biological data. HL collaborated in patient care and collecting data. JS supervised the manuscript. JF revised the manuscript and gave final approval for its submission. The order in which the names appear is based on the fraction of the total work performed. We are indebted to George von Knorring for his assistance in the preparation of the manuscript.

Disclosures

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Potential implications for clinical practice

- Low doses and high doses of aprotinin seem to have similar effects in reducing transfusion requirements, and either may be used in clinical practice.

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