Does aprotinin influence the inflammatory response to cardiopulmonary bypass in patients?

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Objectives: Aprotinin has been shown to have anti-inflammatory properties, but its effects on the inflammatory reaction to cardiopulmonary bypass remain controversial. This prospective, randomized, double-blind study evaluated the influence of aprotinin on various blood markers of inflammation during and after cardiopulmonary bypass.

Methods: Sixty male patients underwent coronary artery bypass grafting. The patients were randomized into 3 groups: a placebo group, a second group receiving 2,000,000 KIU of aprotinin followed by an infusion of 500,000 KIU/h and 2,000,000 KIU in the pump prime, and a third group receiving half this dosage. Measurements of tumor necrosis factor, interleukin 6, interleukin 8, interleukin 10, endotoxin, histamine, complement factors, prekallikrein, and prostaglandin D₂ were obtained at baseline, 30 minutes after study drug loading, 10 minutes after the beginning of cardiopulmonary bypass, before the end of bypass, 4 hours after bypass, and on the first and second postoperative days.

Results: Aprotinin had no significant effect on any of these parameters. As expected, aprotinin reduced early blood loss in both treated groups.

Conclusions: These results indicate that aprotinin at doses currently used to reduce blood loss has no significant influence on the systemic inflammatory response during moderate hypothermic cardiopulmonary bypass in human subjects, as assessed by the mediators measured in this study.

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Cardiopulmonary bypass (CPB) is associated with a significant inflammatory reaction characterized by complement activation, liberation of endotoxin, cellular activation, and release of cytokines and other mediators.¹⁻³ Chemotactic cytokines, especially interleukin (IL) 8, play a major role in the activation of the inflammatory cascade. Activation of the contact system leads to generation of kallikrein, which in turn leads to plasmin formation and activation of coagulation.⁴ This inflammatory response is associated with an anti-inflammatory response, including the release of IL-10 and tumor necrosis factor (TNF) receptors.⁵ The predominant proinflammatory reaction might contribute to postoperative complications, including the development of postoperative myocardial or other organ dysfunction.⁶ Different strategies for reducing the inflammatory reaction have been proposed, including the administration of corticosteroids,⁷ the use of leukocyte depletion filters,⁸ the use of heparin-coated surfaces in the CPB circuits,⁹ and even the use of off-pump surgical techniques.^{10,11}

Aprotinin, a serine protease inhibitor now largely used to limit perioperative

TABLE 1. Clinical data

	Control group	LD group	HD group
Age (y)	61 ± 11	59 ± 11	62 ± 9
Weight (kg)	82.5 ± 5.9	74.3 ± 8.0*	76.2 ± 8.1*
Height (cm)	172 ± 5	171 ± 6	172 ± 6
No. of grafts	3 ± 1	3 ± 1	3 ± 1
Duration of CPB (min)	100 ± 23	107 ± 23	91 ± 25
Duration of aortic clamping (min)	68 ± 13	69 ± 13	60 ± 17
Amount of blood transfused (mL)	662 ± 274	551 ± 256	417 ± 136

**P* < .05 versus control group.

blood loss, has been proposed as another strategy to limit this inflammatory response by inhibiting some of the proteases involved in the inflammatory activation.^{2,12-16} Plasma concentrations of about 200 KIU/mL of aprotinin should be sufficient to inhibit enzymes like trypsin, plasmin kallikrein, and elastase.¹⁷ In vitro experiments by Soeparwata and colleagues¹⁸ found that aprotinin could limit leukocyte activation after CPB. Gilliland and coworkers,¹⁹ in an in vitro model of CPB, showed that aprotinin reduced the expression of CD18 selectins in granulocytes and monocytes, although it had no effect on monocyte CD11b or IL-8 plasma concentrations. However, in vitro models of CPB exclude many of the factors included in the reaction to CPB, so that extrapolation to clinical CPB should be made with caution. In a porcine model of CPB, Ali and colleagues²⁰ found that aprotinin was able to reduce capillary leakage and vasodilation, and recent reports have suggested that aprotinin might also attenuate the inflammatory reaction to CPB in human subjects.^{2,21-23} Aprotinin administration during CPB has been shown to inhibit the release of TNF- α , IL-8, and IL-6 and to blunt the CPB-induced upregulation of CD11b receptors on neutrophils.^{21,23} However, other investigators have failed to document an influence of aprotinin on proinflammatory cytokine release during CPB.²⁴ In view of these controversial and still fragmented data, the present study was designed as a prospective, controlled, randomized, double-blinded study to evaluate whether aprotinin can reduce the inflammatory response to CPB.

Patients and Methods

After institutional approval by the ethical committee, 60 adult male patients weighing between 50 and 90 kg, who were scheduled for coronary artery bypass graft surgery with at least 2 planned grafts, were enrolled in the study. One patient was withdrawn from the study shortly after the surgical procedure because of perioperative myocardial infarction, requiring immediate reintervention for new bypass grafting. Accordingly, the randomization procedure was extended to a 61st patient. Exclusion criteria were severe left ventricular dysfunction (defined as a left ventricular ejection fraction of <35% or an end-diastolic pressure of >16 mm Hg), autoimmune disease, a presumed or documented infection, preoperative administration of corticosteroids or nonsteroidal anti-

inflammatory medication during the 3 days before the operation, ingestion of drugs with a known antiplatelet effect (eg, aspirin) during the 3 days before the operation, emergency procedure, previous cardiac operation, preoperative hematocrit level of less than 25%, pre-existing liver dysfunction, pre-existing renal dysfunction (defined as serum creatinine level of >1.7 mg/dL), congenital or acquired bleeding disorders, known hypersensitivity to aprotinin, previous exposure to aprotinin, allergic diathesis or atopy, or alcohol or drug abuse.

The 60 patients were divided into 3 groups by means of computerized randomization: (1) a control group that received a placebo solution; (2) a low-dose group that received a priming solution of 1,000,000 KIU of aprotinin (Trasylol; Bayer, Leverkusen, Germany), followed by a continuous infusion of 250,000 KIU/h and 1,000,000 KIU added to the pump prime; and (3) a high-dose group that received a priming solution of 2,000,000 KIU of aprotinin (Trasylol, Bayer), followed by a continuous infusion of 500,000 KIU/h and 2,000,000 KIU added to the pump prime.

The patients were anesthetized with midazolam and sufentanil by using a target-controlled infusion generating a plasma concentration of 100 ng/mL midazolam and a plasma concentration of 2 to 4 ng/mL sufentanil.^{25,26} After induction of anesthesia and administration of pancuronium (0.08 mg/kg), the patients were intubated and ventilated with a fraction of inspired oxygen of 0.50, a respiratory rate of 10 breaths/min, and a tidal volume adjusted to obtain a P_{aCO_2} of 35 to 40 mm Hg. Each patient was monitored with a femoral arterial line and a pulmonary artery catheter. The CPB circuit was primed with a gelatin solution (Haemacel; Hoechst, Brussels, Belgium), and the flow was adjusted to 2.4 L · min⁻¹ · kg⁻¹. An infusion of phenylephrine (Neo-Synephrine; Sanofi, Colomiens, France) or sodium nitroprusside (Nitriate; L'Arguenon Int, Paris, France) was administered when necessary to maintain a mean arterial pressure of between 60 and 90 mm Hg during CPB. Arterial blood gases were managed with alpha-stat. Anterograde cardioplegia was obtained with a cold crystalloid solution of 800 mL. The patients were cooled to moderate hypothermia of 28°C. Anticoagulation was achieved by using a bolus dose of 300 IU/kg of porcine heparin (Natrium Heparine; B. Braun Medical, Jaen, Spain) and additional boluses when necessary to maintain an activated clotting time with kaolin of greater than 480 s. After CPB, heparin activity was reversed with protamine (Protamine ICN1000; Sanico, F. Hoffmann-La Roche AG, Kaiseraugst, Switzerland), assuming a heparin half-life of 60 minutes. No corticosteroids were administered.

Figure 1. Time course of TNF- α , IL-6, IL-8, and IL-10 in the 3 groups of patients. *CTRL*, Control; *LD*, low dose; *HD*, high dose.

Transfusion requirements and blood loss were recorded. Thoracic drainage volume was recorded every 6 hours until the drain was removed.

Blood samples were obtained from the arterial line at baseline (before induction of anesthesia), 30 minutes after study drug loading, 10 minutes after the start of CPB, before the end of CPB, 4 hours after CPB, on the first postoperative day (POD1), and on the second postoperative day (POD2). Measurements included TNF- α , IL-6, IL-8, IL-10, endotoxin, histamine complement factors (C1q, C3d, C3, C4, C4a, and C5a), prekallikrein, and prostaglandin D₂ (PGD₂).

After immediate blood centrifugation at 4°C at 3000 rpm for 10 minutes, the plasma was frozen at -80°C. Cytokines were measured by using immunoassays: TNF- α , IL-6, IL-8, and IL-10 were measured with EASIA kits (Medgenix Diagnostics, Fleurus, Belgium). Endotoxin was determined with the Limulus Amebocyte Lysate test (Endosafe, Charleston, SC). Complement factors C4a and C5a were measured with radioimmunoassays (Biotrak; Amersham International, Buckinghamshire, United Kingdom), and complement factors C1q, C3, and C4 were mea-

sured with a nephelometer analyzer (BNII; Behring Dade & Behring, Marburg, Germany). Prekallikrein levels were determined by using a functional test on the basis of the activation of prekallikrein by the presence of an excess of factor XIIA (KABI-Vitrum, Sweden). PGD₂ was measured with an ELISA method (Cayman Chemical, SPBIO, France).

Sample size was calculated on the basis of the available data on IL-6 increase during cardiac operations, expecting a mean increase of about 700 pg/mL with a relative variation of 800% in the placebo group.⁵ On the basis of a relevant reduction to 100 pg/mL with aprotinin, an accepted type 1 error probability α value of .05 (2-sided), an accepted power 1- β value of .80, and the assumption that IL-6 was log normal distributed, a sample size of 20 patients in each group was necessary. Statistical comparison was done in 2 steps. First, comparison of high-dose treatment with placebo was performed; when this was significant, the low dose was compared with placebo. The data were analyzed by means of covariance analysis for repeated measurements. Variables not normally distributed were analyzed after log transformation. All statistical analyses were performed by Lincoln Systems (Boulogne-Billancourt, France).

TABLE 2. Time course of endotoxin, histamine, and complement factors in the 3 groups of patients

	Group	Baseline	Drug + 30 min	End CPB	CPB + 10 min	CPB + 4 h	POD1	POD2
Endotoxin (pg/mL)	CTRL	4.5 (2.2-6.9)	6.1 (2.3-31.5)	14.0 (5.0-44.5)	35.5 (15.7-79.4)	20.3 (10.0-75.0)	9.7 (4.2-38.0)	5.7 (2.8-22.6)
	LD	4.4 (2.1-9.8)	6.2 (2.8-14.5)	11.7 (5.3-27.7)	23.3 (12.5-43.7)	18.2 (9.2-44.2)	9.4 (5.4-15.7)	5.9 (3.4-16.6)
	HD	3.8 (1.8-19.0)	5.7 (3.2-21.8)	10.2 (6.1-30.7)	18.9 (5.6-43.9)	18.0 (6.0-43.1)	9.4 (3.8-32.4)	6.0 (3.7-18.0)
Histamine (mg/dL)	CTRL	204 (135-424)	294 (141-644)	76 (0-202)	68 (0-260)	141 (90-224)	184 (122-258)	209 (143-251)
	LD	284 (138-735)	287 (169-659)	114 (0-200)	67 (0-211)	213 (122-501)	191 (128-504)	190 (125-443)
	HD	230 (164-408)	278 (165-480)	84 (39-173)	79 (0-141)	170 (71-394)	198 (126-309)	224 (116-347)
C1q (mg/dL)	CTRL	10 (8-16)	9 (7-14)	5 (3-6)	5 (4-7)	7 (5-8)	4 (3-7)	6 (5-11)
	LD	9 (7-11)	9 (6-11)	4 (4-6)	5 (5-7)	7 (5-9)	6 (4-7)	6 (4-9)
	HD	9 (7-12)	8 (7-11)	5 (4-5)	6 (5-7)	8 (5-9)	5 (4-8)	6 (3-9)
C3 (mg/dL)	CTRL	147 (117-210)	127 (94-159)	59 (48-83)	68 (60-92)	83 (68-105)	73 (60-118)	97 (79-159)
	LD	130 (100-167)	109 (83-144)	55 (44-73)	69 (52-92)	85 (62-108)	77 (57-96)	98 (81-123)
	HD	130 (112-190)	117 (100-159)	60 (51-75)	76 (61-92)	92 (63-132)	77 (54-112)	88 (65-145)
C3d (mg/dL)	CTRL	4.5 (1.9-10.5)	4.7 (1.4-7.1)	2.0 (0.7-4.5)	3.3 (1.2-5.3)	3.8 (1.6-6.2)	2.4 (1.3-4.9)	2.6 (1.5-6.1)
	LD	3.8 (1.6-7.7)	3.1 (1.5-7.4)	1.8 (0.9-3.9)	3.0 (1.4-4.8)	2.9 (1.6-5.2)	2.3 (1.0-3.9)	3.2 (1.4-6.3)
	HD	5.4 (1.4-10.7)	4.7 (1.0-7.2)	2.2 (0.7-3.9)	3.5 (1.1-5.0)	3.3 (1.6-6.3)	2.6 (0.8-5.2)	3.0 (1.0-6.7)
C4 (mg/dL)	CTRL	36 (28-65)	32 (21-50)	12 (8-18)	15 (10-21)	22 (14-32)	18 (12-31)	25 (16-60)
	LD	36 (22-56)	29 (18-45)	12 (8-14)	14 (9-19)	20 (15-33)	19 (13-28)	24 (16-37)
	HD	31 (22-56)	27 (20-46)	13 (9-15)	15 (11-20)	22 (15-33)	21 (12-32)	22 (14-40)
C5a (mg/dL)	CTRL	14 (10-34)	16 (10-20)	15 (10-27)	14 (10-26)	13 (10-41)	10 (10-28)	11 (10-39)
	LD	13 (10-21)	12 (10-22)	10 (10-26)	13 (10-32)	13 (10-21)	10 (10-29)	10 (10-23)
	HD	14 (10-25)	11 (10-30)	14 (10-27)	12 (10-22)	10 (10-20)	10 (10-25)	11 (10-36)

Values are given as means (25th-75th percentile). CTRL, Control; LD, low dose; HD, high dose.

Results

Demographic data are given in Table 1. There was no significant difference among the 3 groups of patients regarding age, height, duration of CPB, aortic crossclamping, and number of grafts, but the patients in the aprotinin groups had a lower body weight than those in the placebo group. There were no significant differences in inflammatory markers and hemodynamic data at baseline.

The time course of blood cytokine levels is shown in Figure 1. TNF- α and IL-8 levels increased transiently in the first hours after CPB. IL-6 levels markedly increased after CPB and remained increased on POD1 and POD2. There were no significant differences among the 3 groups in TNF- α , IL-6, or IL-8 levels. IL-10 increased to greater than baseline values at the end of CPB. A tendency toward a higher value in the placebo group was noted, but because IL-10 levels were less than the detection threshold in numerous patients, no meaningful statistical analysis could be done on this value.

Endotoxin levels increased during CPB and returned to baseline levels on POD1 and POD2 (Table 2). They were higher in the placebo group than in the treated groups before the end of CPB, but this was due to one outlying patient who had a maximum endotoxin increase that was 10 times higher than that seen in any other patient (560 pg/mL). This patient had an otherwise uncomplicated postoperative course.

The time course of the complement factors was identical in all 3 groups (Table 2). C4a levels decreased during CPB and returned to baseline values after CPB (Figure 2). C5a levels were stable during the whole procedure. C1q, C3,

C3d, and C4 levels decreased during CPB and increased after CPB, without returning to their baseline values (Table 2).

Prekallikrein levels decreased sharply at the beginning of CPB and then increased, without returning to baseline levels. Prekallikrein levels were greater in the high-dose group than in the other groups only at CPB plus 10 minutes (Figure 2).

PGD₂ levels transiently increased at the end of CPB. Except for a lower PGD₂ level in both treatment groups than in the placebo group at CPB plus 10 minutes, there were no significant differences among the 3 groups (Figure 3).

Aprotinin resulted in a reduction in blood loss on the day of the operation (Figure 3), although overall, there was no significant difference in the amount of red blood cells or other blood products transfused (Table 1). No blood products were transfused during CPB. There were no differences between the groups in the amounts of heparin or protamine administered. There was no allergic reaction to aprotinin, and no significant differences among the 3 groups in the numbers of adverse events, including the most commonly reported: atrial fibrillation, renal function abnormalities, and hypoxia.

Discussion

The present placebo-controlled, randomized, double-blinded trial indicates that aprotinin at doses used clinically has no significant effect on the release of several inflammatory markers in human subjects during CPB. High-dose, as well as low-dose, aprotinin has been extensively shown to

Figure 2. Time course of prekallikrein, PGD₂, and C4a in the 3 groups of patients. CTRL, Control; LD, low dose; HD, high dose.

reduce bleeding during and after CPB.^{16,27} In our study also aprotinin was able to effectively reduce blood losses after cardiac operations. Although aprotinin did not significantly influence transfusion requirements, there was a trend toward

increased transfusion in the control group. Importantly, because transfusion increases cytokine release, the control group would thus have had increased cytokine levels enhancing any apparent anti-inflammatory effects of aprotinin, but even allowing for this, we were unable to notice any differences in inflammatory response among the groups.

There are many proposed markers of the inflammatory response in human subjects, and we selected to measure several of the key mediators as a guide to the degree of this response. Although some studies have suggested a relationship between mediator levels and clinical outcomes, this link has not been firmly established.^{28,29} In addition, the degree of mediator release might be influenced by the preoperative status of the patient and by genetic differences, such as variations in the *TNF* allele.³⁰

Endotoxin is often released during CPB, perhaps from the underperfused gut.^{31,32} In our study endotoxin levels were lower in the treated than in the placebo group, but these differences were due to one outlier in the placebo group who had a 10-fold maximum increase compared with that seen in the other patients.

TNF- α is synthesized as a membrane-bound precursor that has to be cleaved to yield soluble TNF proteins, and this might represent a possible site of action for aprotinin to inhibit TNF- α release. Hill and colleagues²¹ showed that even low doses of aprotinin reduced TNF- α liberation during CPB in human subjects in the same manner as methylprednisolone. Our study showed a significant increase in TNF- α in all 3 groups but no effect of aprotinin on this process. Recently, another prospective, randomized trial³³ in 200 patients undergoing CPB studied the effects of high-dose aprotinin and heparin-coated circuits. Fifty patients were randomized to one of 4 groups: heparin-coated circuit with or without aprotinin and uncoated circuit with or without aprotinin. The authors of that study³³ found that aprotinin had no effect on levels of TNF- α , IL-6, or IL-8.

Aprotinin has been shown in some studies to reduce other cytokine levels during CPB, namely IL-6 and IL-8, but these reports are from the studies reporting a lesser release of TNF- α , the major stimulus for release of other cytokines.^{21,34} In our study aprotinin failed to inhibit the increase in IL-6, IL-8, and IL-10 during and after CPB. The doses used in our study were either equivalent (low-dose group) or higher (high-dose group) than those in the study by Hill and colleagues,²¹ so that dosage does not explain the different findings. However, the study by Hill and colleagues included only 8 patients in each group. Ashraf and coworkers²⁴ and Gott and associates³⁵ found that aprotinin, although it inhibited fibrinolysis, had no effect on IL-8 levels in human subjects. IL-10, a major anti-inflammatory cytokine, is also released during CPB, especially after the administration of methylprednisolone,^{5,36,37} and Hill and

Figure 3. Blood loss in the 3 groups of patients. *CTRL*, Control; *LD*, low dose; *HD*, high dose.

colleagues³⁷ noted that this IL-10 release was enhanced in patients given aprotinin.

Other aspects of the inflammatory response in addition to cytokine mediators, including cell factors, complement, CD11/CD18, and elastase, are also activated during CPB² and could thus potentially be influenced by aprotinin. Complement activation during CPB is a possible pathway leading to an increased formation of TNF- α .³⁸ Gott and coworkers³⁵ compared 4 anti-inflammatory strategies in CPB, and in their study only heparin-bonded circuits, but not aprotinin, decreased complement activation. In our study complement system activation was indicated by the decrease in C1q, C3, and C4 and the increase in C3a after CPB, but aprotinin had no effect on this process. Activation of the contact system leads to the formation of kallikrein,³⁹ which can itself activate the complement system, the bradykinin system, and proinflammatory cytokine release. Although aprotinin is able to inhibit the action of kallikrein,⁴⁰ it had no consistent effect on prekallikrein levels in our study.

Mast cells are also activated during CPB, and postoperative dysrhythmias in children might be due to histamine liberation,⁴¹ but in our study there was no increase in histamine levels during CPB. PGD₂ is liberated by activated mast cells,⁴² but we found no studies that measured PGD₂ during and after CPB. Although PGD₂ levels were significantly lower at the end of CPB in treated patients, these differences might be due to chance because they were transient and not dose related.

One might argue that higher doses of aprotinin than that used in the high-dose regimen in our study might be more efficient in suppressing the inflammatory response to CPB. However, the doses we used are those routinely used in clinical practice. Moreover, the high doses of aprotinin used

in this study have been shown to result in plasma concentrations of about 200 KIU/mL, which are sufficient to inhibit enzymes, such as trypsin, plasmin kallikrein, and elastase.^{17,21}

In conclusion, although aprotinin has some potential for inhibition of inflammatory pathways, this prospective, randomized, double-blinded trial was unable to demonstrate a significant effect of even high doses of aprotinin on a number of key inflammatory mediators during CPB.

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