Aprotinin Improves Functional Outcome but Not Cerebral Infarct Size in an Experimental Model of Stroke During Cardiopulmonary Bypass

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BACKGROUND: Aprotinin, a nonspecific serine protease inhibitor, has been used to decrease bleeding and reduce the systemic inflammatory response after cardiopulmonary bypass (CPB). Studies have variably linked aprotinin administration with both improved as well as adverse cerebral consequences after cardiac surgery. We designed this study to determine whether an antiinflammatory dose of aprotinin could improve the histologic and functional neurologic outcome in a rat model of focal cerebral ischemia during CPB.

METHODS: After surgical preparation, the animals were randomized into 2 groups: an aprotinin group (60,000 kIU/kg IV) and a control group (0.9% NaCl IV). Normothermic CPB was performed for 60 minutes during which time a partial overlapping 60 minutes of right middle cerebral artery occlusion was induced. Cytokines (tumor necrosis factor-α, interleukin [IL]-1β, IL-6, and IL-10) were measured at baseline, the end of CPB, then 2 and 24 hours after CPB. On postoperative day 3, the animals underwent functional neurologic testing and histologic assessment of cerebral infarct volume.

RESULTS: There was a reduction in systemic inflammation in the aprotinin group compared with the control group, demonstrated by lower levels of IL-1β ($P = 0.035$) and IL-6 ($P = 0.047$). The aprotinin group also had a better functional neurologic performance (median [interquartile range]: aprotinin 27 [8] vs control 32 [6]; $P = 0.042$). However, there was no difference in cerebral infarct volume (aprotinin 306 [27] mm^3 vs control 297 [52] mm^3; $P = 0.599$).

CONCLUSIONS: In this experimental model of stroke occurring during CPB, aprotinin decreased the systemic inflammatory response to CPB. Although there was no difference in the cerebral infarct volume, there was a small improvement in the short-term functional neurologic outcome in the aprotinin group. (Anesth Analg 2010;111:38–45)

Stroke and neurocognitive dysfunction after cardiac surgery remain significant causes of perioperative morbidity and mortality.[1] As with other organ systems, the brain is exposed to many deleterious events during cardiac surgery and cardiopulmonary bypass (CPB). These events include transient periods of low perfusion pressure (particularly during CPB), as well as cerebral macro- and microembolism, both leading to ischemia that can be modulated by other intraoperative and postoperative factors.[2] Despite the many studies in this area, the pathophysiology of brain ischemia in the CPB setting is not clearly understood. Interestingly, one of the unique aspects of cerebral injury during cardiac surgery is the superimposition of a pronounced systemic inflammatory response that occurs as a result of CPB.[3] Consequently, the inflammatory response has become an increasingly important potential therapeutic target for the prevention of cerebral injury in cardiac surgical patients.

Various maneuvers to decrease CPB-induced systemic inflammation have focused on the development of new technologies, such as heparin-coated CPB systems, whereas others have centered on pharmacological therapies. Among antiinflammatory therapies, aprotinin was, until relatively recently, one of the commonly used drugs during cardiac surgery. This nonspecific serine protease inhibitor has had mixed results in affecting cerebral outcome after CPB. For example, Levy et al.[4] demonstrated in a post hoc analysis from a large study that patients receiving aprotinin had a significant reduction in the incidence of stroke after cardiac surgery compared with patients receiving placebo. In addition, in a subsequent retrospective analysis of patients considered at high risk for stroke during cardiac surgery, Frumento et al.[5] demonstrated a reduced risk for stroke in those receiving aprotinin. Despite these clinical studies demonstrating the potential benefit of aprotinin, no evaluation of aprotinin with stroke as a primary outcome had been performed in any prospective randomized trial.

More recently, Mangano et al.[6] elaborated on potential adverse effects of aprotinin in their observational study of 4374 patients. In that study, in addition to demonstrating increased renal complications associated with its use, patients who received aprotinin had a significantly higher rate of cerebrovascular complications ($P < 0.001$). In the largest prospective randomized trial of aprotinin examining bleeding, the BART (Blood Conservation Using Antifibrinolytics: A Randomized Trial) investigators found a

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significant reduction in bleeding, but an overall mortality risk with aprotinin compared with other antifibrinolytics.[7] The BART investigators did not find a higher rate of stroke with aprotinin compared with tranexamic acid (2.5% with aprotinin vs 3.7% with tranexamic acid; relative risk 0.78 (0.45–1.35, 95% confidence interval). In laboratory models, studies evaluating aprotinin for neuroprotection have similarly been inconclusive. In an in vitro analysis of cerebellar neuronal cultures, aprotinin had a dose-related neuroprotective effect.[8] Also, high-dose aprotinin improved brain metabolic recovery after transient global ischemia in rats and after CPB in piglets.[9,10] In contrast, in a rat study of global and focal brain ischemia, no direct neuroprotection from the use of a high dose of aprotinin was demonstrated.[11]

Although aprotinin is no longer available for clinical use, the relevance of the potential neurologic effects of kallikrein inhibition remains. Indeed, there are at least 2 other highly potent kallikrein inhibitors (CU-2010 and ecallantide) in current clinical development.[12–14] Therefore, the objective of this study was to determine whether an antiinflammatory dose of aprotinin could improve the histologic and functional neurologic outcome in an experimental model combining focal cerebral ischemia with CPB.

METHODS

The Duke University Institutional Animal Care and Use Committee approved this study, and all procedures met the National Institutes of Health guidelines for animal care.[15]

Surgical Procedures

Male Wistar rats (Harlan, Indianapolis, IN), 12–14 weeks of age and body weight of 275–300 g, were fasted 12 hours overnight but allowed free access to water before experiments. The animals were anesthetized in a chamber with 3% isoflurane in 50% oxygen/50% nitrogen. The trachea was then intubated (16-gauge catheter), the lungs were mechanically ventilated (Harvard® Rodent Respirator; Harvard Apparatus, Boston, MA), and the isoflurane concentration was reduced to 1.5% to 2.0%. The ventilatory variables were adjusted to attain an arterial carbon dioxide tension of 36 to 42 mm Hg. A needle thermistor was inserted pericranially (in the left temporal muscle adjacent to the skull) and this temperature was servo-controlled (YSI 400 series thermistor and 73 ATA Indicating Controller; YSI, Yellow Springs, OH) with both forced-air and surface-heating systems to the defined target temperature (37.5°C). The rectal temperature (core temperature) was also monitored and recorded.

The animals were then prepared for CPB as follows. The right superficial epigastric artery was cannulated (PE-10 Intramedic polyethylene tubing; Becton Dickinson, Sparks, MD) for continuous monitoring of mean arterial blood pressure and for arterial blood gas sampling (IL-1306 blood gas analyzer; Instrumentation Laboratories, Lexington, MA). Heparin (150 IU) was administered through this cannula, both to keep the cannula from being occluded from thrombus during the surgical dissection and also to allow for systemic anticoagulation for CPB. The ventral tail artery was dissected, cannulated (20-gauge, 1.16-in. IV catheter—Insyte-W™; Becton Dickinson, Sandy, UT), and used as the arterial inflow for the CPB circuit. Using a PE-50 catheter (Intramedic polyethylene tubing), the tail vein was cannulated for drug and fluid administration. After this, a cervical anterior midline incision was made and the right external jugular vein was identified and isolated for later cannulation. The right common carotid artery was then surgically prepared for middle cerebral artery occlusion (MCAO). Using blunt dissection, its branches were identified and isolated using 5-0 silk. The external carotid artery was ligated remote from its origin and the proximal end was temporarily occluded with a microsurgical aneurysm clip thereby allowing subsequent intraluminal filament insertion. The jugular vein was incised and a modified multiorificed cannula (4.5F Desilets-Hoffman Pediatric Introducer; Cook Medical, Bloomington, IN) was inserted and advanced into the right heart for use as the venous return cannula of the CPB circuit.

The animals were then allowed to stabilize for 15 minutes after which MCAO was performed by insertion of the filament (4-0 mononylon with the tip heat blunted and coated with silicone) through the external carotid artery stump. The filament was advanced until resistance indicated (from previous surgical experience) that the tip had occluded the circle of Willis at the origin of the middle cerebral artery (19–20 mm from carotid bifurcation).[16] After onset of the MCAO, mechanical ventilation was stopped and normothermic (37.5°C) CPB was initiated.

The CPB circuit was a custom-made apparatus consisting of a reservoir to collect the venous blood from the external jugular vein and was located 12 cm below the heart level creating a gravity gradient for drainage. The venous blood collected in the reservoir drained to a peristaltic pump (Masterflex®; Cole-Parmer Instrument Co., Vernon Hills, IL) via silicone tubing (1.6-mm internal diameter, Tycon®; Cole-Parmer Instrument Co.) and was subsequently pumped through a small custom-made membrane oxygenator. This oxygenator was constructed of 2 Plexiglas® shells holding 3 layers of diffusion membrane (9.8 cm^2) bound together in a crosswise fashion. This device was manufactured to permit the exchange of gases and delivery of volatile anesthetic (isoflurane) to the oxygenated blood. In addition, the membrane oxygenator was attached to a heat exchanger through which a continuous flow of warmed (37°C) water ensured the maintenance of blood temperature. Sequentially, in line with the membrane oxygenator, the blood was directed to the tail arterial inflow cannula. The CPB flow was continuously monitored with a flowprobe located within the circuit (2N806 flowprobe and T208 volume flowmeter; Transonics Systems, Ithaca, NY).

The CPB circuit was primed with 10 mL of whole blood obtained from 1 heparinized donor rat (100 IU heparin, IV, per animal) collected through a silicone catheter inserted in the right external jugular vein. Isoflurane anesthesia (1.2%) was maintained during CPB, and atracurium (0.3 mg/kg IV repeated every 25 minutes) was administered (after establishing in pilot experiments that unparalyzed animals under isoflurane anesthesia did not exhibit escape behavior in response to noxious stimuli) to prevent any diaphragmatic movement.

CPB (flow rate: 160–180 mL/kg/min), adjusted to maintain a minimal venous reservoir blood level, was maintained for 60 minutes. The MCAO filament was removed after 60 minutes of focal cerebral ischemia. After weaning from CPB,

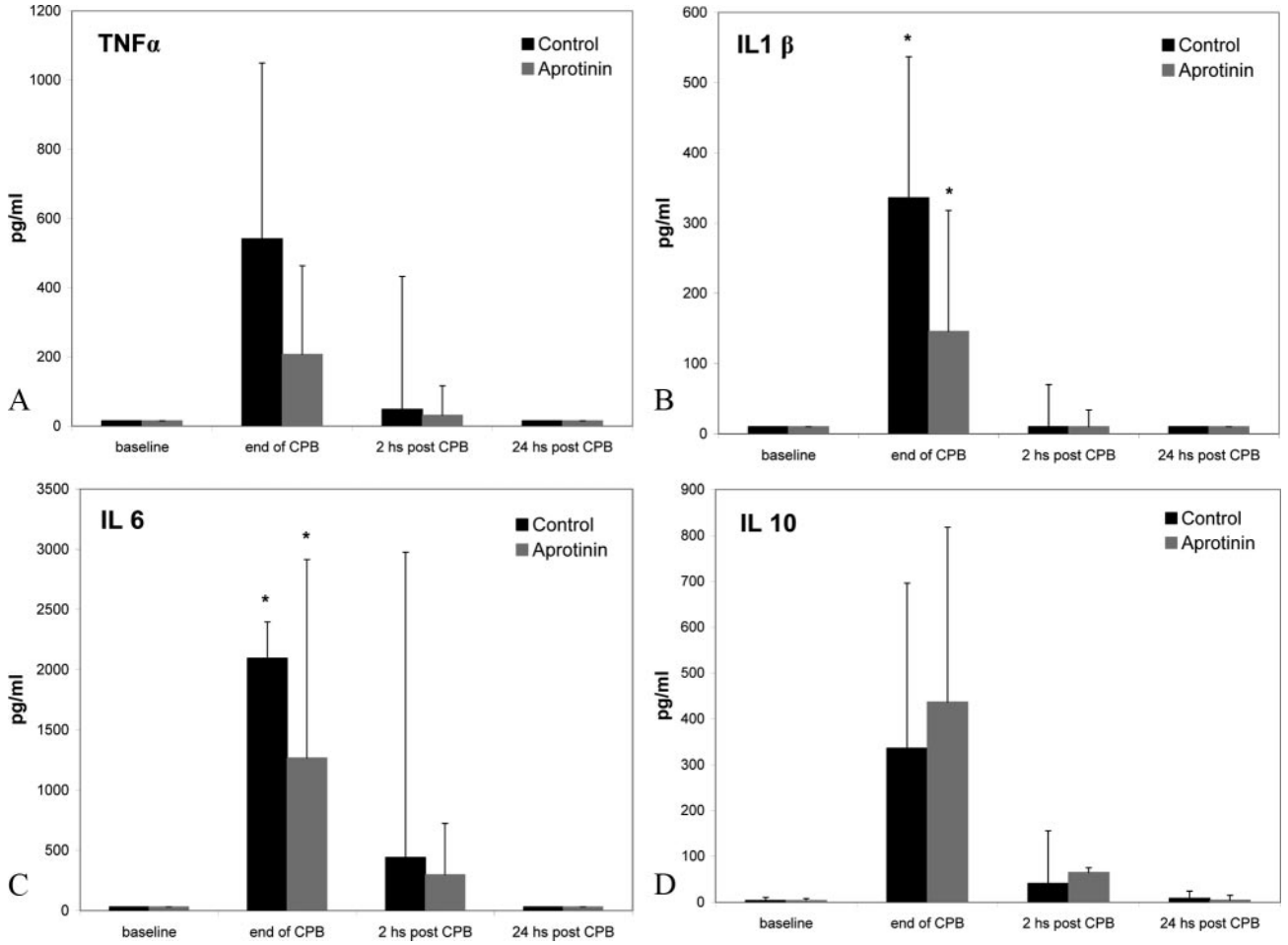

Figure 1. Serum cytokine levels for aprotinin-treated animals and controls at each study time point. A, tumor necrosis factor (TNF)-α; B, interleukin (IL)-1β; C, IL-6; and D, IL-10. There was an increase in all serum cytokines in both experimental groups at the end of cardiopulmonary bypass (CPB) and 2 hours after CPB compared with baseline levels. The levels of TNF-α tended to be lower in the aprotinin group compared with controls at the end of CPB but not at any other time point (baseline, $P > 0.999$; TNF-α end of CPB, $P = 0.141$; TNF-α 2 hours after CPB, $P = 0.443$; TNF-α 24 hours after CPB, $P > 0.999$). Although not different at baseline, serum levels of IL-1β and IL-6 were lower at the end of CPB in the aprotinin group compared with controls (IL-1β baseline, $P = 0.654$; IL-1β end of CPB, $P = 0.035$; IL-1β 2 hours after CPB, $P = 0.798$; IL-1β 24 hours after CPB, $P > 0.999$) (IL-6 baseline, $P = 0.654$; IL-6 end of CPB, $P = 0.047$; IL-6 2 hours after CPB, $P = 0.949$; IL-6 24 hours after CPB, $P = 0.886$). There was no difference in IL-10 levels between the aprotinin and control groups (IL-10 baseline, $P = 0.654$; IL-10 end of CPB, $P = 0.406$; IL-10 2 hours after CPB, $P = 0.337$; IL-10 24 hours after CPB, $P = 0.617$). The values are expressed as median (interquartile range). *$P < 0.05$ for between-group comparisons.

the animals were maintained under general anesthesia allowing for an additional 120 minutes of ventilatory support; no vasopressors were required. Immediately at the end of CPB, the right external jugular multiorificed cannula was exchanged for a PE-50 catheter (Intramedic polyethylene tubing) that was implanted to collect serial blood samples after animal recovery (24 hours after CPB). All other catheters were removed; the wounds were infiltrated with 1% lidocaine and then closed with silk sutures. After the experiment, the rats were housed in individual cages with a controlled environmental temperature (25°C) and oxygen supply (fraction of inspired oxygen = 0.5) for 24 hours, after which they were returned to their previous cage.

Experimental Design

After surgical preparation, the animals were randomized to 1 of 2 groups: aprotinin group ($n = 8$) or control group ($n = 8$) (Fig. 1). Ten minutes before MCAO, the aprotinin animals received an initial loading dose of 60,000 kIU/kg IV administered over 10 minutes using an infusion pump. When the loading dose was completed, a continuous aprotinin infusion was started (15,000 kIU/kg/h IV) and maintained throughout CPB. The control animals received an equivalent volume of isotonic saline (0.9% NaCl). The operator was blinded to group assignment and provided with coded syringes containing aprotinin or saline.

Serum Cytokine Analyses

Four blood samples were collected from the right external jugular vein for cytokine measurements at the following time points: before CPB (baseline), at the end of CPB, then 2 and 24 hours after CPB. The blood samples were immediately centrifuged (4°C) and the supernatant was frozen (−80°C) for later analyses. Serum tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and IL-10 were analyzed using multiplexed sandwich enzyme-linked immunosorbent assay

(SearchLight® Proteomic Arrays; Pierce Biotechnology, Woburn, MA). Results were expressed as picograms per milliliter (pg/mL) and sensitivity for detection was 3.1 pg/mL for tumor necrosis factor-α, 3.1 pg/mL for IL-1β, 6.3 pg/mL for IL-6, and 0.4 pg/mL for IL-10.

Coagulation Cascade Analysis

Blood was collected into tubes containing sodium citrate (3.2%; 1:10 volume) at 4 time points (baseline, at the end of CPB, then 2 and 24 hours after CPB) for analysis of thrombin-antithrombin III complex (TAT) and D-dimer. The blood samples were immediately centrifuged (4°C) with the plasma stored (−80°C) for later batch analyses. TAT levels were used as a measure of thrombin generation and D-dimer was used as an indicator of fibrinolysis. TAT levels were detected by sandwich enzyme-linked immunosorbent assay microtiter plate (Behringwerke AG, Marburg, Germany), following the manufacturer's instructions. Although these kits measure human TAT (sensitivity of detection for human TAT 1.0–4.1 μg/L), the polyclonal antibodies in this enzyme-linked immunosorbent assay have been shown to be cross-reactive with rat TAT complexes.[17]

Quantitative determination of rat-specific D-dimer was measured by a homogeneous latex particle–based immunoassay using the MDA D-dimer kit (bioMérieux, Durham, NC). Results were expressed as fibrinogen equivalent units/mL. Normal range of detection is 0.000 to 0.349 fibrinogen equivalent units/mL and values >0.5 fibrinogen equivalent units/mL are positive.

Neurologic Evaluation

On postoperative day 3, the rats underwent functional neurologic evaluation (by an observer blinded to the group assignments) using 2 different numerical scales. The first test scored the animals between 0 to 18 points using simple motor tasks to identify neurologic deficits (in which 18 points corresponds to a normal behavior).[18] This functional testing assesses the following: spontaneous activity = no movement (0) to normal behavior (3 points); motor symmetry in all limbs = no movement at the left side (0) to normal behavior (3 points); motor symmetry in the forelimbs = no movement (0) to normal motor symmetry (3 points); climbing = falls trying to climb (1 point) to normal climbing (3 points); body proprioception = no response at left side (1 point) to symmetrical response (3 points); and the response to vibrissae touch = no response on the left side (1 point) to symmetrical response (3 points).

The second neurologic test was adapted from 4 previously published scores[19–22] and has been validated in other MCAO models in rats.[23] By observing motor skills and sensory responses, this neurologic scoring system was graduated from 0 (normal behavior) to 48 (complete absence of motor and sensory responses). The tests observe the following: general status of motor behavior (spontaneous activity in 5 minutes: normal [0] to not observed [4]; body symmetry: normal [0] to extreme asymmetry [4]; gait: normal [0] to does not walk [4]); simple motor tests (front limb symmetry: normal [0] to no limb movement [4]; circling at the bench top: not present [0] to pivoting [4]; circling by holding tail: normal [0] to does not advance [4]; hind limb placement: normal [0] to no placement [2]); complex motor tests (vertical screen climbing: normal [0] to slides immediately [4]; beam walking: walks to the other end [0] to falls immediately [4]); sensory responses (forelimb touch with a needle: normal [0] to no withdrawal [2]; hindlimb with a needle: normal [0] to no withdrawal [2]; trunk touch with a needle: symmetrical response [0] to response absent bilaterally [4]; vibrissae touch: normal response [0] to response absent bilaterally [4]; face touch: normal [0] to no withdrawal [2]).

Cerebral Infarct Volume Evaluation

After neurologic evaluation, the animals were anesthetized with 5% isoflurane and decapitated. The brain was immediately removed, immersed in methylbutane, and frozen at −35°C, after which it was sectioned for infarct volume measurement. Briefly, coronal sections were made (20-μm thickness) and 4 sequential slices were collected and slide mounted. The next 33 sections were discarded and then a new 4-slice cycle was begun. The process began with the most rostral slice where the cerebral infarct was evident and continued throughout the extent of the infarct. The sections were stained with hematoxylin and eosin. The brain sections were then digitized (1289 × 960 matrix of 210-μm^2 pixel units) using an image analysis system (M2 Turnkey System; Imaging Research, St. Catharines, ON, Canada). The infarct boundaries were separately delineated for the cortical and subcortical structures by an observer blinded to group assignment. Infarct volumes (mm^3) were computed as running sums of infarct area multiplied by the known interval (e.g., 660 μm) between sections over the extent of the infarct expressed as an orthogonal projection.

Statistical Analysis

Normally distributed physiological variables and arterial blood gases data were expressed as mean ± SD and differences between groups were compared using the unpaired Student *t* test. For serum cytokines, TAT, D-dimer concentrations, which were not normally distributed, the nonparametric Mann-Whitney *U* test was applied as appropriate. Also, cerebral infarct volumes and neuroscores were compared between groups using the Mann-Whitney *U* test. The nonparametric data were expressed as median (interquartile range). A P value <0.05 was considered significant.

RESULTS

Physiological values are shown in Table 1. There were no significant differences between groups in the arterial blood gas results or other physiological variables.

Results of the proinflammatory cytokines are shown in Figure 1, A–C. There were no differences in cytokine levels between groups at baseline. Blood levels of IL-1β (P = 0.035) and IL-6 (P = 0.047) were lower in the aprotinin group compared with the control group at the end of CPB. Levels of the antiinflammatory cytokine IL-10 trended toward a higher level in the aprotinin group compared with the control group at the end of CPB but this difference was not different (P = 0.406; Fig. 1D). There were no other differences in cytokine levels between groups at any of the other postoperative testing periods.

Serum TAT level results are shown in Figure 2A and serum D-dimer results are presented in Figure 2B. There were no differences between groups in serum TAT levels at

Table 1. Physiological Values

Variables	Control (n = 8)	Aprotinin (n = 8)	P value
Body weight (g)	298 ± 16	310 ± 12	0.113
MAP (mm Hg)			
Baseline	72 ± 3	75 ± 3	0.120
30 min CPB	74 ± 5	72 ± 6	0.563
60 min CPB	74 ± 4	73 ± 5	0.663
30 min after CPB	68 ± 4	68 ± 8	0.836
120 min after CPB	69 ± 7	69 ± 5	0.873
Pericranial temperature (°C)			
Baseline	37.4 ± 0.1	37.4 ± 0.1	0.148
30 min CPB	37.0 ± 0.3	37.0 ± 0.4	0.690
60 min CPB	37.2 ± 0.2	37.3 ± 0.1	0.363
30 min after CPB	37.3 ± 0.2	37.3 ± 0.1	0.936
120 min after CPB	37.4 ± 0.1	37.4 ± 0.1	0.743
Hb (g/dL)			
Baseline	16 ± 2	16 ± 1	0.531
30 min CPB	13 ± 1	12 ± 1	0.655
60 min CPB	13 ± 1	12 ± 2	0.346
30 min after CPB	12 ± 1	11 ± 1	0.715
120 min after CPB	11 ± 1	11 ± 1	0.757
Hct (%)			
Baseline	39 ± 2	39 ± 2	0.830
30 min CPB	31 ± 3	30 ± 5	0.348
60 min CPB	31 ± 4	30 ± 5	0.358
30 min after CPB	28 ± 3	28 ± 3	0.999
120 min after CPB	26 ± 2	28 ± 3	0.580
Glucose (mg/dL)			
Baseline	79 ± 9	83 ± 6	0.391
30 min CPB	90 ± 14	102 ± 10	0.077
60 min after CPB	120 ± 23	117 ± 9	0.688
CPB flow (mL/kg/min)			
30 min	179 ± 11	179 ± 9	0.179
60 min	173 ± 6	175 ± 8	0.373

CPB = cardiopulmonary bypass; MAP = mean arterial blood pressure; Hb = hemoglobin; Hct = hematocrit.
Values are mean ± SD. P value <0.05 was considered significant.

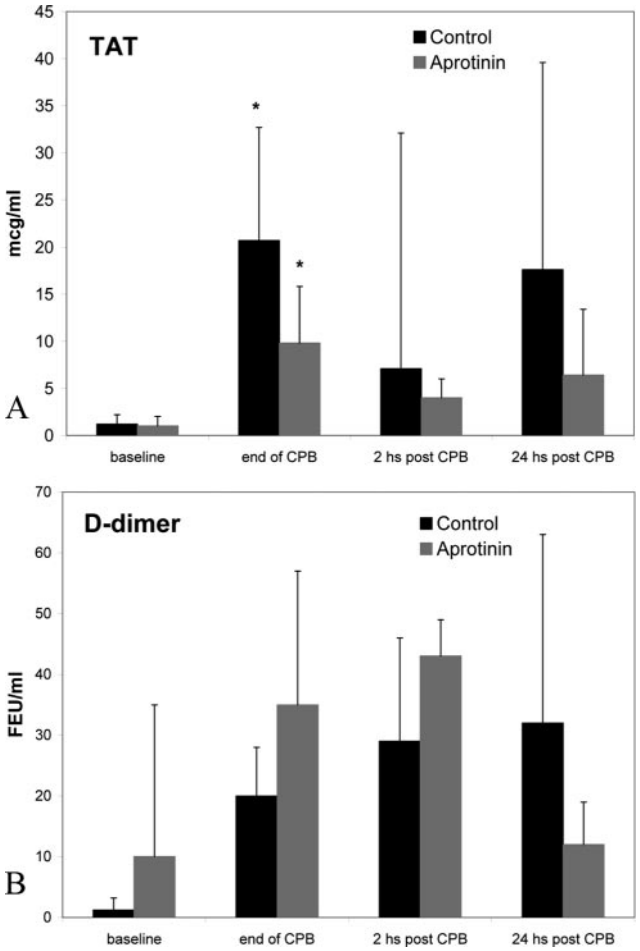

Figure 2. Thrombin-antithrombin III complex (TAT) (A) and D-dimer serum concentrations (B) for aprotinin-treated animals and controls at each study time point. TAT levels showed an increase throughout the experiment in both groups compared with baseline. Compared with controls, TAT levels were lower in the aprotinin group at the end of cardiopulmonary bypass (CPB) but not at any other time point (TAT baseline, P = 0.932; TAT end of CPB, P = 0.035; TAT 2 hours after CPB, P = 0.227; TAT 24 hours after CPB, P = 0.293). There was no difference between groups in D-dimer concentration at any time point (D-dimer baseline, P = 0.603; D-dimer end of CPB, P = 0.310; D-dimer 2 hours after CPB, P = 0.318; D-dimer 24 hours after CPB, P = 0.060). The values are expressed as median (interquartile range). FEU = fibrinogen equivalent units. *P < 0.05.

baseline. TAT serum concentrations were lower in the aprotinin group at the end of CPB compared with the control group (P = 0.035) but there were no differences between groups at any other study time point. Fibrinolytic activity, as measured by D-dimer concentrations, increased during the course of CPB compared with baseline in both groups. Serum D-dimer levels tended to be lower 24 hours after CPB in the aprotinin group compared with the control group (P = 0.060), although this was not statistically different compared with the control group. There were no other differences in D-dimer levels between groups at any other study time point.

Neurologic function scores are represented in Figure 3. Functional neurologic outcome using the 18-point scoring system trended toward improvement in the aprotinin group compared with the control group (P = 0.058). Using the separate 48-point neuroscore, in which zero corresponds to normal behavior (Fig. 3B), the aprotinin group was improved compared with the control group (P = 0.042).

Brain infarct volume results are shown in Figure 4. There was no difference in total cerebral infarct volume between groups (aprotinin, 306 [27] mm^3 vs control, 297 [52] mm^3; P = 0.599) (Fig. 4A). There was no difference between the aprotinin and the control groups in cortical infarct volumes (aprotinin, 223 [38] mm^3 vs control, 238 [29] mm^3; P = 0.752) (Fig. 4B) and subcortical infarct volume (aprotinin, 83 [24] mm^3 vs control, 78 [25] mm^3; P = 0.528) (Fig. 4C).

DISCUSSION

Central nervous system injuries manifesting either as stroke or neurocognitive dysfunction continue to be major contributors to morbidity and mortality in patients undergoing surgery with CPB.[1,24,25] Although the pathophysiology of brain injury after cardiac surgery is not fully understood, most injuries are believed to result from cerebral embolization and cerebral hypoperfusion (either regional or global) that may both be exacerbated by CPB-related inflammation.[3,26]

The systemic inflammatory response is well recognized during CPB in both experimental and clinical settings.[3,27] This inflammatory response is characterized by an imbalance between pro- and antiinflammatory cytokine production. The

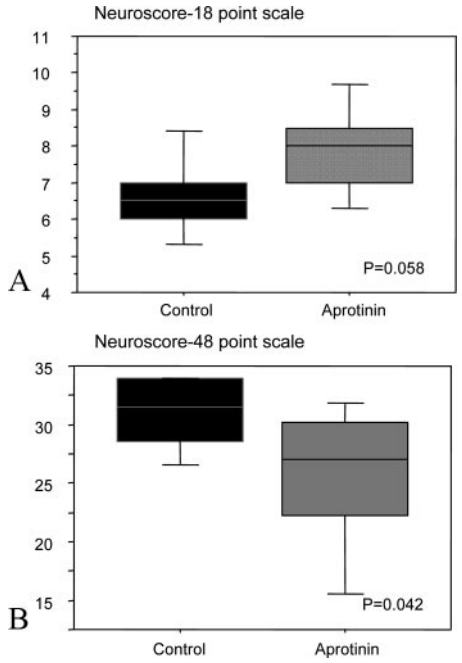

Figure 3. Neurologic assessment results from postoperative day 3 for the aprotinin-treated animals and controls. The neurologic outcome was analyzed using 2 different point scales. The first numerical scale (A), in which 18 points represents a normal neurologic examination, showed a trend ($P = 0.058$) toward a better neurologic outcome for the aprotinin-treated animals compared with the controls. The second numerical testing (B) used a 48-point scale, in which zero corresponds to normal behavior, showed better performances in the aprotinin group compared with the control group ($P = 0.042$). The values are expressed as median (interquartile range).

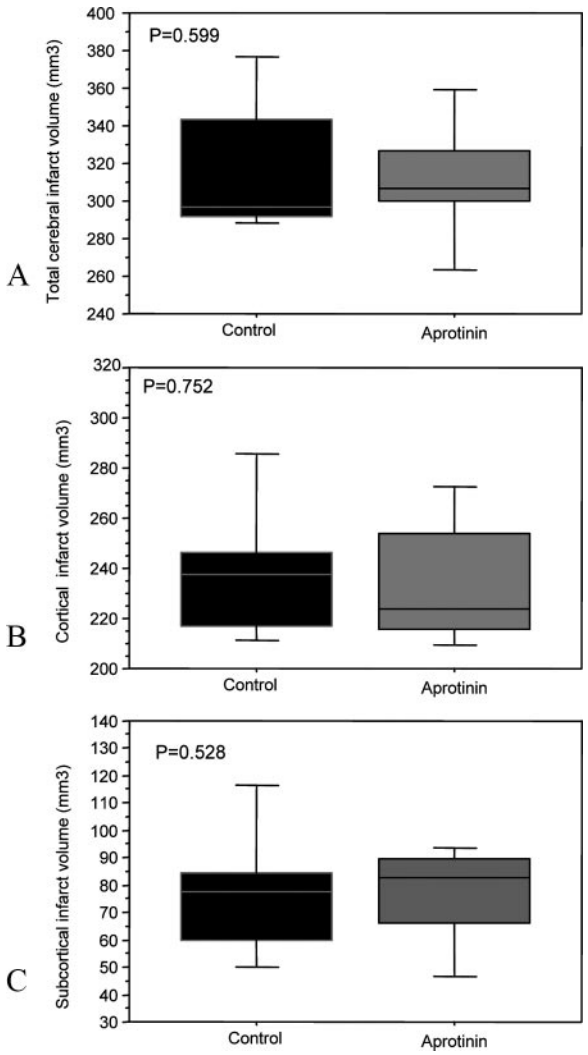

Figure 4. The cerebral infarct volume for aprotinin-treated animals and controls. The infarct volume was analyzed both as the total cerebral infarct volume (A), and for the cortical (B) and subcortical (C) infarct volumes. There was no difference between groups in the volume of cerebral infarction for each comparison. The values are expressed as median (interquartile range).

proinflammatory balance is coupled with leukocyte activation and degranulation, along with platelet activation, all of which further contribute to inflammation. This inflammatory response has been correlated with respiratory dysfunction, renal failure, and myocardial and neurologic injuries.[28,29]

To decrease morbidity and mortality related to cardiac surgery, various antiinflammatory strategies have been used. Among pharmacological approaches, aprotinin has shown variable effects on both the coagulation and inflammatory cascades during CPB.[30,31] Although the full molecular mechanism for aprotinin's action is not completely understood, it has been demonstrated to possess antiinflammatory effects, in part related to kallikrein inhibition, decreased neutrophil activation, reduced vascular permeability, and resultant decreased systemic cytokine production.[31] Aprotinin also inhibits the activation of circulating leukocytes, reducing the transmigration of these cells into tissues.[30]

Until relatively recently, aprotinin had been used in cardiac surgery to decrease bleeding and consequently the need for blood transfusion. Early studies directed at elucidating its blood loss–sparing effects demonstrated a decrease in stroke rate as a secondary finding in patients who received aprotinin versus placebo during coronary artery bypass graft surgery.[4,5] The mechanisms for the potential decrease in stroke incidence with aprotinin are not clearly known. Although its effects on inflammation were implicated, others suggest that this neurologic benefit was more directly related to its properties in diminishing bleeding, consequently leading to less cardiotomy suction blood returning to the venous reservoir that itself has been shown to be related to cerebral emboli.[32,33] Nonetheless, the enthusiasm for a potential beneficial effect of aprotinin for reducing perioperative brain injury has been tempered by a large observational study by Mangano et al.[6] that found an increase in stroke in patients who received aprotinin compared with aminocaproic acid.

Standardized conditions of an experimental laboratory offer a unique opportunity to study the complexities of CPB-induced effects on the coagulation and inflammatory systems superimposed on central nervous system injury. In this study, the administration of aprotinin resulted in an improvement in functional neurologic outcome compared with control animals. At the same time, we observed a decrease in the systemic inflammatory response to CPB with significant reductions in IL-1β and IL-6, and a trend

toward an increase in the antiinflammatory cytokine IL-10. Similar inflammatory data have been reported in humans undergoing cardiac surgery who received aprotinin.[34] Nonetheless, we did not observe a reduction in cerebral infarct size in animals receiving aprotinin versus controls.

We speculate that the decrease in systemic inflammatory response with aprotinin was related to better functional neurologic outcome in our study. In previous experiments, we had examined the effect of aprotinin on cerebral ischemia outside the setting of CPB and did not demonstrate any direct neuroprotective effects.[9] However, in CPB, we speculate that the ability of aprotinin to reduce the overall inflammatory milieu was responsible for the beneficial functional neurologic effect.

An interesting finding in this study is the reduction in thrombin generation, as seen by the lower formation of TAT levels in aprotinin-treated animals. This might also be a reason why inflammation was attenuated. There is considerable cross-talk between thrombin and inflammation, and higher thrombin levels may increase inflammation in the setting of CPB.[35]

We used a combination of 2 neurologic scoring systems to comprehensively evaluate functional neurologic stroke outcome in this experimental model. Using a more robust neurologic evaluation, the animals that received aprotinin demonstrated improved overall performance compared with controls. In the 48-point score system, 4 functions were used to evaluate overall neurologic outcome (general status, simple and complex motor deficits, and sensory deficit). This scoring system has been validated in rats submitted to focal ischemia whereby both simple and complex motor assessments have demonstrated a robust association with infarct size.[23]

There were several limitations in this study, including the dose of aprotinin. The dose used in our experiments is at least as high as the amount considered a full dose for pediatric cardiac patients (40,000–50,000 kIU/kg).[36,37] Furthermore, the dose in this study has been used in rats submitted to global and focal ischemia.[11] However, we did not measure aprotinin levels, but with an estimated 33% blood volume dilution factor in our animals after initiation of CPB, and in the absence of any ultrafiltration that might alter the levels, we assumed that the 60,000 kIU/kg initial loading dose was likely sufficient to maintain blood aprotinin levels in an effective therapeutic range. Although studies have shown that plasma levels to inhibit plasmin (50 kIU/mL) are lower than those necessary to inhibit kallikrein (200 kIU/mL), a plasma level of 200 kIU/mL has been considered effective in decreasing fibrinolysis, inhibiting kallikrein and thrombin generation.[31,37] Our results are in accordance with other literature related to the use of aprotinin leading to inhibition of proinflammatory cytokine levels and thrombin generation after CPB. Consistent with the reductions in inflammatory markers, we saw a significant reduction in TAT levels at the end of CPB, and a trend ($P = 0.06$) toward a reduction in D-dimer levels at 24 hours after CPB despite seeing no effect in the D-dimer levels at the other time points.

Another limitation of our study is related to the stroke model itself. MCAO was produced in these animals via filament insertion through the carotid artery. This may represent a different mechanism of stroke than that occurring in clinical CPB where it is more likely caused by embolic material consisting of thrombus and/or atherosclerotic material. The model we used was of reversible cerebral ischemia (with the insertion and subsequent removal of the occlusive filament) whereas in a clinical setting it may be more likely to be permanent cerebral ischemia. The mechanism by which aprotinin acts on these differing mechanisms could conceivably be different. It is not clear whether our results would have been similar if we had used a permanent occlusion model.[38]

A further potential limitation relates to the timing of our neurologic and histologic assessments. There are 2 reasons we chose postoperative day 3 to analyze the cerebral infarct size in our protocol. First, the systemic inflammation measured using plasma cytokines was undetectable 24 hours after surgery, making it less likely that systemic inflammation had a direct effect on the brain 3 days after surgery. In addition, neutrophil infiltration in the ischemic hemisphere has been shown as early as 1 hour after reperfusion, increases significantly by 6 to 12 hours,[38] and peaks between 24 and 72 hours,[39] making it unlikely that a later assessment period would have had an effect on outcome related to inflammation. In other studies of MCAO in rats, the infarct volume changed significantly between 6 and 72 hours, with little change after that.[40] However, any conclusion of the effect that aprotinin could have had on outcomes if it was administered for a longer time period is speculative.

In summary, in this experimental model of stroke occurring during CPB, aprotinin decreased the systemic inflammatory response from CPB. Although there was no difference in the cerebral infarct volume, aprotinin use was associated with improved short-term functional neurologic outcome.

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