Efficacy of ε-Aminocaproic Acid and Aprotinin on Leukocyte–Platelet Adhesion in Patients Undergoing Cardiac Surgery

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Received from the University of Texas Southwestern Medical Center, Dallas, Texas; Dallas Veterans Affairs Medical Center, Dallas, Texas; and Yale University, New Haven, Connecticut. Submitted for publication March 7, 2003. Accepted for publication July 11, 2003. Supported by a New Investigator Award from the American Society of Anesthesiologists, New Orleans, Louisiana, October 13–17, 2001.

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Background: The administration of aprotinin during cardiopulmonary bypass (CPB) is hypothesized to decrease activation of leukocytes and platelets and possibly reduce their adhesion. Although ε-aminocaproic acid (EACA) shares the ability of aprotinin to inhibit excessive plasmin activity after CPB, its effect on leukocyte and platelet activation and leukocyte–platelet (heterotypic) adhesion is largely unknown. This study was performed to determine the relative effectiveness of the antifibrinolics, aprotinin and EACA, at reducing leukocyte and platelet activation and leukocyte–platelet conjugate formation in patients undergoing CPB.

Methods: Thirty-six patients scheduled to undergo cardiac surgery with CPB were randomized in a double-blind fashion to receive EACA, aprotinin, or saline (placebo). Markers of plasmin activity (D-dimer concentrations), platelet activation (CD62P), leukocyte activation (CD11b), and leukocyte–platelet adhesion (monocyte–neutrophil–platelet conjugates) were measured before, during, and after CPB.

Results: Platelet CD62P (P-selectin), monocyte CD11b, and monocyte–platelet conjugates were all significantly increased compared with baseline in the saline group during and after CPB. Despite equivalent reductions in D-dimer formation in patients receiving EACA (P < 0.0001) and aprotinin (P < 0.0001), decreases in platelet CD62P and monocyte CD11b expression were incomplete (not significantly different from saline control). In contrast, peak monocyte–platelet conjugate formation was significantly reduced by both EACA (P = 0.026) and aprotinin (P = 0.039) immediately after CPB.

Conclusions: EACA seems to be as effective as aprotinin at reducing peak monocyte–platelet adhesion after CPB. Furthermore, inhibition of excessive plasmin activity seems to influence monocyte–platelet adhesion. The findings suggest that monocyte–platelet conjugate formation may be a useful marker of monocyte and platelet activation in this clinical setting.

Cardiopulmonary bypass (CPB) triggers multiple stimuli that lead to leukocyte and platelet activation and subsequent adhesion. Activated platelets rapidly externalize CD62P (also known as P-selectin), an adhesive ligand for phagocytes, from platelet α granules to the external cell surface. In a similar fashion, when leukocytes are activated, the adhesion-promoting β3 integrin CD11b (Mac-1) is up-regulated on the surface membrane. After cell activation and subsequent expression of these adhesive molecules, leukocyte–platelet (heterotypic) conjugates are formed by the binding of CD62P/P-selectin to the leukocyte P-selectin glycoprotein ligand 1 receptor, as well as via fibrinogen bridging between glycoprotein IIb/IIIa and leukocyte CD11b. Leukocyte–platelet interaction may augment cellular activation by promoting the release of reactive oxygen species, proinflammatory cytokines, and thromboxane. Increased concentrations of circulating leukocyte–platelet conjugates have been associated with increases in myocardial injury.

Aprotinin has been used in cardiac surgery to suppress fibrinolysis and preserve hemostasis primarily by inhibiting a broad spectrum of serine proteases including plasmin and kallikrein. However, recent attention has focused on its potential antiinflammatory properties in patients undergoing CPB because both plasmin and kallikrein amplify the inflammatory response by activating key components of the complement cascade. Aprotinin inhibits plasmin activity by binding directly to the active center of plasmin. Inhibition of plasmin activity is crudely assessed by measuring D-dimer concentrations, a cleavage product of plasmin-induced digestion of cross-linked fibrin.

Aprotinin has been shown to significantly reduce D-dimer concentrations during and after CPB as well as attenuate increases in several markers (tumor necrosis factor α, cytokines, elastase, and leukocyte adhesion molecule expression) of the inflammatory response. Although some studies have shown that aprotinin reduces both platelet and leukocyte activation during CPB, the precise mechanisms and proteases responsible for platelet and leukocyte activation have not been delineated.

ε-Aminocaproic acid (EACA) is an antifibrinolytic lysine analog that inhibits plasmin activity by preventing plasminogen from binding to fibrin, resulting in suppression of fibrinolysis. However, little is known about the inhibitory effects of EACA in relation to the inflammatory response. Several studies have suggested that plasmin may have physiologic functions beyond fibrinolysis. Plasmin stimulates monocytes and induces neutrophil aggregation, implying some proinflammatory activity. There are currently no prospective, randomized, placebo-controlled trials in which patients undergoing CPB...
received aprotinin or EACA. Such a study would help to
assess the impact of excessive plasmin activity on leuko-
cyte and platelet activation and heterotypic adhesion
during CPB.

We hypothesized that aprotinin, given its ability to
inhibit multiple serine proteases, would be more effect-
vative at significantly reducing leukocyte-platelet activa-
tion and leukocyte-platelet adhesion than EACA in pa-
tients undergoing CPB. We performed a prospective,
randomized, double-blind, placebo-controlled trial to
determine the effectiveness of these two antifibrinolytic
agents at attenuating the markers of plasmin activity
(D-dimer), platelet (CD62P) activation, leukocyte
(CD11b) activation, and leukocyte-platelet conjugate
formation in patients undergoing CPB.

Materials and Methods

Study Design

After approval by the Dallas Veterans Affairs Subcommit-
tee on Human Use (Research and Development Commit-
tee, Dallas, Texas) and written informed consent, 36 pa-
tients undergoing cardiac surgery with CPB were enrolled.
Patients were randomized in a doubled-blind fashion to (1)
EACA (100-mg/kg loading dose, 30-mg·kg⁻¹·h⁻¹ infusion
rate, and 5 g in the pump prime), (2) full-dose aprotinin
(2 × 10⁶-KIU loading dose, 5 × 10⁶-KIU/h infusion rate,
and 2 × 10⁶ KIU in the pump prime), or (3) saline. Similar
volumes of study material were administered with bolus
doses, pump primes, and infusion. Exclusion criteria in-
cluded a recent history of using corticosteroids, anticoagu-
lation therapy, intravenous tissue plasminogen activators,
or streptokinase (within 5 days of surgery); documented plate-
let or coagulation abnormalities; and use of dipyridamole or
any other antiplatelet therapy other than aspirin (within 2
weeks of surgery). In addition, patients were excluded if
their serum creatinine concentration was greater than 2.0
mg/dl, their ejection fraction was less than 30%, or they had
a history of adverse reaction to aprotinin or EACA.

Anesthesia Technique

Preoperative medications, including β blockers, nitrates,
and antiarrhythmics, were continued until sur-
gery. After sedation with 2–5 mg intravenous midazo-
lam, routine monitors and a radial arterial catheter were
placed. After induction with 0.3 mg/kg etomidate,
5–10 µg/kg fentanyl, and 1 mg/kg rocuronium, a pulmo-
nary artery catheter was inserted via the right internal jugular
vein. Anesthesia was maintained with 0.4–1.0% inhaled isoflurane and 25–50 µg/kg intravenous fenta-
nyl. A propofol infusion, 25–50 µg·kg⁻¹·min⁻¹, was
started at the beginning of rewarming and continued
into the postoperative period for sedation.

Cardiopulmonary Bypass

Cardiopulmonary bypass was performed using a mem-
brane oxygenator (model CE0123; Gish, Irvine, CA) with
nonpulsatile flow using a centrifugal pump (Biomedicus,
Eden Prairie, MN). The CPB circuit was primed with 1.8 l
lactated Ringer’s solution; 100 ml albumin, 25%;
44.6 mEq sodium bicarbonate; and 50 g mannitol. Myo-
cardial protection included moderate hypothermia (28°–
32°C) with antegrade and retrograde sanguineous (4:1
blood:crystalloid) cardioplegia every 10–20 min. Perfu-
sion flow rates were maintained at 2·1·min⁻¹·mm⁻²
during hypothermia and at 2.5·1·min⁻¹·mm⁻² during
normothermia. The α-stat method was used for pH man-
agement during hypothermic CPB. Anticoagulation was
achieved with bovine heparin and monitored to achieve an
initial kaolin activated clotting time of 480 s. A Hep-
con system (Medtronic, Minneapolis, MN) was used for
heparin and protamine titration. Temperature was mon-
itored via a bladder thermistor, and separation of bypass
was initiated at 36.5°C. Reversal and the presence of
residual heparin were monitored with a protamine titra-
tion protocol. Kaolin activated clotting time levels were
documented, and the total amounts of heparin and pro-
tamine used were recorded.

Immunofluorescence and Flow Cytometry

Radial artery blood samples were collected (1) at base-
line (before induction), (2) at the end of hypothermic
CPB (before warming), (3) after the termination of CPB
(after heparin reversal by protamine), and (4) 24 h after
CPB. Blood was drawn into a polypropylene syringe, and
 aliquots were transferred immediately into sterile K₃
EDTA Vacutainers (Becton-Dickinson, San Jose, CA) or
12 × 75-mm Falcon tubes, with the latter containing paraformaldehyde in phosphate-buffered saline (1% final
concentration).

For determination of monocyte and neutrophil CD11b
expression, 100 µl whole blood from the K₃ EDTA
Vacutainer was labeled with 20 µl phycoerythrin-anti-
CD11b (Becton-Dickinson) and 20 µl fluorescein isothio-
cy cyanate conjugated-anti-CD14 (Becton-Dickinson). The
samples were vortexed gently and incubated for 30 min
in the dark at room temperature (20°–25°C). The eryth-
rocytes were then lysed with 2 ml of 1 × FACS Lysing
Solution (Becton-Dickinson). The samples were then
centrifuged (500g for 5 min), and the pellet was washed
with ice-cold phosphate-buffered saline containing 0.1%
sodium azide and resuspended in 500 µl paraformalde-
hyde in phosphate-buffered saline (1% final) with 0.1%
sodium azide. The samples were stored at 4°C until
analyzed later on the day of collection. Irrelevant iso-
typic control antibodies were used in each patient to
determine nonspecific fluorescence.

Samples for monocyte and neutrophil CD11b were
analyzed using a FACStar Plus flow cytometer and
CellQuest software (Becton-Dickinson). The monocyte

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and neutrophil populations were identified on the basis of their characteristic forward and side scatter, and further definition of the monocytes was achieved by specific CD14 staining. A minimum of 5,000 monocyte events and 10,000 neutrophil events were acquired for each sample. The relative density of CD11b fluorescence on monocytes and neutrophils was measured as mean fluorescence intensity and was expressed for each patient as a percentage of their baseline (preinduction) value.

For enumeration of platelet CD62P (P-selectin) expression and leukocyte–platelet conjugates, whole blood was immediately fixed after sampling in 1% paraformaldehyde (final concentration) in phosphate-buffered saline. After incubation for 60 min at 4°C, the paraformaldehyde was neutralized (3:1 vol:vol) with Tris-glycine in Tyrodes-HEPES buffer with 2% bovine serum albumin (THBSA). The sample was then shipped at 4°C by overnight carrier to New Haven, Connecticut. On arrival, samples were confirmed to be at 4°C and then immediately washed twice in THBSA before antibody labeling. In extensive preliminary studies using whole blood samples that were split for immediate versus postshipping analysis, these methods were shown to preserve both CD62P antigenic expression and formation of leukocyte–platelet conjugates. Anti-CD41 (Immunotech, Miami, FL) was used to identify single platelets (for subsequent measurement of the percentage of platelets expressing CD62P) and for labeling of platelets bound to monocytes and neutrophils (enumeration of leukocyte–platelet conjugates). Anti-CD62P, anti-CD14, and anti-CD45 were obtained from Becton-Dickinson. As above, isotypic control antibodies were used to set thresholds for specific binding of CD62P for the percentage of activated platelets and for binding of anti-CD41 to leukocytes for the percentage of leukocyte–platelet conjugates. All samples were labeled and analyzed on a FACSCT (Becton-Dickinson) as previously detailed. Briefly, anti-CD41 and anti-CD62P were combined in one aliquot to determine the percentage of activated platelets, and anti-CD41, anti-CD45, and anti-CD14 were combined in a separate sample to determine the percentage of neutrophils and monocytes with bound platelets.

**D-dimer Concentrations**

Arterial blood samples were collected directly into polypropylene syringe at (1) baseline (before anesthetic induction), (2) normothermic CPB before separation from CPB, and (3) after the termination of CPB (after heparin reversal by protamine) and were then immediately transferred into a 3.2% Na-citrate–buffered Becton-Dickinson brand Vacutainer. D-dimer concentrations were determined using a turbidimetric immunoassay (Beckman Coulter, Miami, FL). All standards and samples were assayed in duplicate.

**Results**

**Patient Characteristics**

Data were analyzed from a total of 35 patients randomized to EACA (n = 11), aprotinin (n = 12), or saline (n = 12). One subject randomized to the EACA group was excluded from the analysis. An episode of profound hy-
Table 1. Patient Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Saline</th>
<th>Aprotinin</th>
<th>EACA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Age, yr</td>
<td>65 ± 8</td>
<td>62 ± 8</td>
<td>64 ± 9</td>
</tr>
<tr>
<td>Male sex</td>
<td>12 (100)</td>
<td>12 (100)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>30.50 ± 3.58</td>
<td>28.53 ± 6.62</td>
<td>31.51 ± 3.87</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>46 ± 13</td>
<td>43 ± 12</td>
<td>56 ± 13*</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking (&lt;3 months)</td>
<td>2 (17)</td>
<td>4 (33)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>8 (67)</td>
<td>10 (83)</td>
<td>11 (92)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>11 (92)</td>
<td>9 (75)</td>
<td>9 (75)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (42)</td>
<td>5 (42)</td>
<td>8 (67)</td>
</tr>
<tr>
<td>Previous cardiac history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent myocardial infarction (&lt;21 days)</td>
<td>1 (8)</td>
<td>1 (8)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>Canadian Cardiovascular Score (angina)</td>
<td>3.00 ± 0.85</td>
<td>2.56 ± 0.88</td>
<td>3.00 ± 0.89</td>
</tr>
<tr>
<td>New York Heart Association functional class</td>
<td>1.75 ± 0.87</td>
<td>2.00 ± 0.94</td>
<td>1.83 ± 0.83</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>12 (100)</td>
<td>12 (100)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>Intravenous heparin</td>
<td>1 (8)</td>
<td>1 (8)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>7 (58)</td>
<td>9 (75)</td>
<td>10 (83)</td>
</tr>
<tr>
<td>β Blockers</td>
<td>9 (75)</td>
<td>9 (75)</td>
<td>9 (75)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>5 (42)</td>
<td>9 (75)</td>
<td>8 (67)</td>
</tr>
</tbody>
</table>

Values are mean ± SD unless otherwise indicated. Values in parentheses represent percentages of each group.
* P < 0.05 when compared with aprotinin.

ACE = angiotensin converting enzyme; EACA = ε-aminocaproic acid.

D-dimer concentrations in all three groups increased significantly (P < 0.0001) during normothermic CPB (compared with baseline) and peaked immediately after CPB. D-dimer concentrations during and immediately after CPB were significantly attenuated in the EACA (P < 0.0001) and aprotinin (P < 0.0001) groups compared with the saline group. Furthermore, D-dimer concentrations were not different between patients receiving EACA and aprotinin.

Cell Counts

Perioperative cell counts are shown in table 4. Platelet, monocyte, and neutrophil counts decreased during CPB in all three treatment groups. Platelet counts remained depressed at the end of CPB and at 24 h after CPB in all of the groups. Monocyte counts also remained below preinduction (baseline) values at the end of CPB; by contrast, neutrophil counts at the end of CPB in all of the groups were increased from baseline. A dramatic rebound in monocyte counts was noted at 24 h after CPB, and these values were significantly (P < 0.05) higher than the baseline values in all of the groups. Compared with saline controls, no significant differences were revealed in the aprotinin or EACA groups with respect to platelet, neutrophil, or monocyte counts at any time during or after CPB. However, monocyte counts were significantly (P = 0.02) better preserved in the EACA group compared with aprotinin immediately after CPB.

Platelet Activation

The percentage of circulating activated (P-selectin-expressing) platelets increased from baseline by more
than 200% and peaked during hypothermic CPB (before warming) in all treatment groups (fig. 1). Although the percent of P-selectin-positive platelets increased substantially in the saline group, neither of the antifibrinolytics significantly attenuated the increase in platelet P-selectin expression during CPB surgery. Immediately after CPB, circulating activated platelet concentrations decreased but still remained significantly increased from baseline in the saline (P = 0.01) and aprotinin (P = 0.042) groups but not in the EACA groups (P = 0.062). There were no significant differences in the percentage of platelets expressing P-selectin between treatment groups at any time during or after CPB.

Monocyte and Neutrophil Activation

Leukocyte activation was assessed by mean surface fluorescence of CD11b on monocytes (fig. 2) and neutrophils before, during, and after CPB. Monocyte CD11b expression increased significantly from baseline during CPB in the saline and aprotinin groups (P = 0.026 and P = 0.022, respectively) but not in the EACA group (P = 0.124). Monocyte CD11b expression reached a peak value in all three treatment groups immediately after CPB (end CPB), and subsequently decreased, reaching baseline values at 24 h after CPB. Although the overall treatment effect of aprotinin and EACA in reducing monocyte CD11b expression did not reach statistical significance, a trend (P = 0.07) was noted in the EACA group. Neutrophil CD11b expression also increased during CPB and reached a peak value at the end of CPB (data not shown). Compared with monocyte CD11b expression in the saline group, neutrophil CD11b expression peaked at lower levels (< 150% of baseline) at the end of CPB, but, like monocyte CD11b, there were no significant differences between treatment groups at any time before, during, or after CPB.

Table 3. D-dimer Concentrations, μg/l

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preinduction</th>
<th>Normothermic CPB</th>
<th>End CPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>143 ± 88</td>
<td>1,020 ± 261</td>
<td>1,082 ± 231</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>159 ± 63</td>
<td>407 ± 229*</td>
<td>448 ± 233*</td>
</tr>
<tr>
<td>EACA</td>
<td>180 ± 110</td>
<td>474 ± 255*</td>
<td>627 ± 287*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Normal range (concentration) less than 500 μg/l.

* P < 0.0001 when compared with saline.

CPB = cardiopulmonary bypass; EACA = ε-aminocaproic acid.
CPB (end CPB) and was again significantly increased from baseline in the saline group (P = 0.002) but not in either the aprotinin or the EACA group. Peak monocyte–platelet conjugate formation was significantly attenuated in both the EACA (P = 0.026) and the aprotinin (P = 0.039) group compared with the saline control group. Although no significant relations (r^2 < 0.1) were found between D-dimer concentrations and any of the markers of platelet and leukocyte activation or adhesion, modest relations between peak monocyte–platelet adhesion and monocyte CD11b (r^2 = 0.34; P = 0.002) and P-selectin (r^2 = 0.37; P = 0.0008) were noted immediately after CPB. Unlike monocyte–platelet conjugate formation during and after CPB, the percentage of neutrophil–platelet conjugates remained relatively constant in the saline group over time, and neither of the treatment groups was significantly different from the saline group at any time point (data not shown).

Discussion

This randomized, double-blind, placebo-controlled study is the first to evaluate the effects of EACA and aprotinin on leukocyte and platelet activation and leukocyte–platelet (heterotypic) adhesion in patients undergoing cardiac surgery. Contrary to our hypothesis, we found that EACA was as effective as aprotinin at attenuating monocyte–platelet conjugate formation during and after CPB, and this was the most significant effect of both antifibrinolytic agents. Furthermore, we found that CPB-induced increases in monocyte–platelet adhesion coincided with increases in markers of both platelet (P-selectin) and monocyte (CD11b) activation and were positively correlated with these markers. Our observations confirm previous work that showed that this heterotypic cellular interaction is related to activation of these cellular subsets and support the notion that monocyte–platelet adhesion/conjugate formation is a meaningful marker of monocyte and platelet activation and may be superior to measuring P-selectin or leukocyte CD11b in patients undergoing CPB.22

In our study, CPB-induced platelet activation was evidenced by increases in P-selectin expression, which reached its peak during hypothermic CPB. P-selectin was significantly increased in all treatment groups and remained increased throughout the first postoperative day. The modest reductions in P-selectin expression, which were achieved with both antifibrinolytic agents, are consistent with previous studies.23–26 The lack of a treatment effect on P-selectin noted in this study may be due to either a limited “platelet-sparing” effect or possibly to loss of circulating P-selectin–expressing platelets to the CPB circuit and/or microcirculation. Exposure of blood to the biomaterial surfaces of the CPB circuit leads to activation of a variety of physiologic systems, including complement, coagulation, and fibrinolysis.15,16 Subsequent generation of complement components (C3a, C5a, and C5b-9)27,28 and activation of serine proteases

**Table 4. Cell Counts**

<table>
<thead>
<tr>
<th></th>
<th>Before Induction</th>
<th>Before Warming</th>
<th>End CPB</th>
<th>24 h After CPB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet count, × 10^5/ml</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>195 ± 44.7</td>
<td>115 ± 37.5*</td>
<td>118 ± 40.8*</td>
<td>112 ± 31.8*</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>186 ± 71.5</td>
<td>95 ± 29.3*</td>
<td>92 ± 30.1*</td>
<td>114 ± 39.1*</td>
</tr>
<tr>
<td>EACA</td>
<td>195 ± 58.6</td>
<td>113 ± 27.1*</td>
<td>106 ± 30.4*</td>
<td>111 ± 17.4*</td>
</tr>
<tr>
<td><strong>Neutrophil count, × 10^5/ml</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4.58 ± 1.22</td>
<td>4.21 ± 3.25</td>
<td>7.59 ± 4.38*</td>
<td>7.45 ± 2.44*</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>4.77 ± 1.22</td>
<td>2.75 ± 1.27</td>
<td>7.17 ± 3.21*</td>
<td>9.71 ± 2.87*</td>
</tr>
<tr>
<td>EACA</td>
<td>5.01 ± 2.17</td>
<td>4.46 ± 2.26</td>
<td>8.63 ± 4.51*</td>
<td>8.15 ± 2.78*</td>
</tr>
<tr>
<td><strong>Monocyte count, × 10^5/ml</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.52 ± 0.28</td>
<td>0.28 ± 0.22*</td>
<td>0.25 ± 0.19</td>
<td>0.75 ± 0.34*</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>0.53 ± 0.31</td>
<td>0.17 ± 0.09*</td>
<td>0.19 ± 0.11*</td>
<td>0.96 ± 0.44*</td>
</tr>
<tr>
<td>EACA</td>
<td>0.48 ± 0.26</td>
<td>0.39 ± 0.26</td>
<td>0.43 ± 0.22†</td>
<td>0.82 ± 0.39*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Normal ranges (platelets: 140–400 × 10^9/ml; neutrophils: 1.6–7.7 × 10^9/ml; monocytes: 0.1–1.0 × 10^9/ml).

* P < 0.05 when compared with preinduction (baseline) values. † P < 0.05 when compared with aprotinin.

CPB = cardiopulmonary bypass; EACA = ε-aminocaproic acid.

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**Fig. 1.** The percentage of platelets expressing CD62P (P-selectin) measured before, during, and after cardiopulmonary bypass (CPB) is shown relative to the percentage of P-selectin–positive platelets at baseline. Measurements are presented as mean ± SEM. † P < 0.05 compared with baseline values. EACA = ε-aminocaproic acid.
Leukocyte CD11b expression reached its peak immediately after CPB and was higher in monocytes than in neutrophils. Higher levels of monocyte CD11b expression may partly explain why the percentage of monocyte–platelet conjugates compared with neutrophil–platelet conjugates. It is also interesting to note that unlike P-selectin, which peaked during hypothermic CPB, peak monocyte and neutrophil activation occurred after protamine administration at the conclusion of CPB. The fact that peak monocyte CD11b expression coincided with peak monocyte–platelet conjugate formation immediately after CPB suggests that CD11b may contribute to leukocyte–platelet adhesion, possibly via fibrinogen bridging to the platelet’s glycoprotein IIb/IIIa receptor. This is consistent with other studies that indicate leukocyte adhesion is in addition mediated by mechanisms other than P-selectin and P-selectin glycoprotein ligand. Unlike previous studies, which showed dramatic increases in neutrophil CD11b after CPB, our study showed only slight increases compared with baseline. It is unclear whether the frequent use of statin therapy and/or angiotensin-converting enzyme inhibitors in our study or some other procedural factor (heparin management, CPB, or others) resulted in lower concentrations of circulating neutrophils expressing CD11b.

The use of antifibrinolytic therapy during CPB had a pronounced effect on reducing monocyte–platelet adhesion. Peak monocyte–platelet adhesion, unlike the neutrophil–platelet subset, was significantly attenuated by both EACA and aprotinin immediately after CPB. It is interesting to note that both antifibrinolytic agents caused similar reductions in monocyte–platelet adhesion despite profound differences in their modes of action. Aprotinin inhibits plasmin activity by binding directly to the active center of plasmin, whereas EACA inhibits by steric/functional hindrance and possibly by releasing α2 antiplasmin. This equivalent effect of EACA and aprotinin on monocyte–platelet conjugate formation and D-dimer concentrations suggests that excessive plasmin activity (especially at the termination of CPB) may be an important contributor to platelet and leukocyte activation and adhesion in patients undergoing CPB. Although no significant correlations were found between D-dimer concentrations and any of the markers of platelet and leukocyte activation or adhesion, this may be because of the possibility that D-dimer concentrations may not be the best marker for evaluating the effects of excessive plasmin activity on these heterotypic cellular interactions. An alternative explanation may be that the blockade of lysine-binding sites by EACA could also prevent plasminogen binding to leukocytes, possibly inhibiting a microenvironment that favors leukocyte–platelet conjugate formation.

The pathophysiologic significance of leukocyte–platelet adhesion in patients undergoing CPB is hypothesized to include targeting of cell populations to areas of vascular injury and amplifying of inflammatory and thrombogenic cellular responses. Cooperative metabolic action and signaling between leukocytes and platelets have been shown to occur when in the presence of one
another and after adhesion.\textsuperscript{9,15,30} Faraday et al.\textsuperscript{37} have demonstrated that platelet agonist-induced aggregation is enhanced in the presence of leukocytes, and others have shown that platelets adherent to leukocytes increase the release of superoxide anions, expression of interleukins (interleukins 1\(\beta\) and 8), monocyte chemotactic protein 1, and thromboxane.\textsuperscript{9,58,59} Although a growing body of evidence suggests that leukocyte–platelet adhesion increases thrombotic risk,\textsuperscript{13,14,22} studies exploring the correlation between monocyte–platelet conjugate formation and in vivo markers of organ injury are needed to establish the clinical relevance of this marker in patients undergoing CPB.

The limitations of this study are similar to those associated with any clinical trial attempting to evaluate a drug effect under a complex set of pathophysiologic conditions. Although our groups were relatively well matched, other covariates may exist that influenced the inflammatory response in a study of this size. This study was not designed to identify the precise mechanism by which EACA or aprotinin alters leukocyte–platelet adhesion, nor was it designed to show a direct correlation between the level of plasmin activity and inhibition of leukocyte–platelet conjugate formation. Finally, optimal antifibrinolytic concentrations and dosing regimens for achieving maximal suppression of excessive plasmin activity during and after CPB have not been established; therefore, the possibility exists that higher concentrations of either agent might have resulted in significantly more inhibition of leukocyte and platelet activation and perhaps a better correlation with fibrinolytic activity (i.e., D-dimer concentrations).

In conclusion, we have demonstrated that EACA is as effective as aprotinin in reducing CPB-induced monocyte–platelet conjugate formation. Although aprotinin and EACA achieved equivalent reductions in D-dimer concentrations during CPB and in the early post-CBP period, the effect of these antifibrinolytic agents on P-selectin and leukocyte CD11b expression was not significant (incomplete blockade). These data support the notion that monocyte–platelet adhesion/conjugate formation is a meaningful marker of monocyte and platelet activation and may be superior to measuring P-selectin or leukocyte CD11b in patients undergoing CPB.

The authors thank all of the members of the Perioperative Cardiovascular Research Team and Special Coagulation/Inflammation Research Laboratory at the Dallas Veterans Affairs Medical Center (Dallas, Texas).

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