Aprotinin But Not ε-Aminocaproic Acid Decreases Interleukin-10 After Cardiac Surgery With Extracorporeal Circulation
Randomized, Double-Blind, Placebo-Controlled Study in Patients Receiving Aprotinin and ε-Aminocaproic Acid

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Background—Extracorporeal circulation induces a systemic inflammatory response, which may adversely affect organ function. One manifestation of this response is increased fibrinolysis. Antifibrinolytic drugs such as aprotinin and ε-aminocaproic acid have been effective in reducing fibrinolysis and blood loss after extracorporeal circulation; however, the effects of antifibrinolytic drugs on proinflammatory and anti-inflammatory mediators are not known. This study examined the effects of aprotinin and ε-aminocaproic acid on plasma levels of proinflammatory [interleukin-6 (IL-6)] and anti-inflammatory [interleukin-10 (IL-10)] cytokines during and after extracorporeal circulation.

Methods and Results—Seventy-two patients undergoing coronary artery bypass grafting with extracorporeal circulation were randomly assigned in a double-blind study to receive high-dose aprotinin, ε-aminocaproic acid, or saline placebo. Plasma levels of IL-6 and IL-10 were measured at 5 time points before, during, and after extracorporeal circulation. In all 3 groups, both IL-6 and IL-10 rose significantly after institution of extracorporeal circulation and remained elevated through the first postoperative day. Compared with saline, aprotinin significantly reduced IL-10 (P = 0.02) and peak IL-6 (P = 0.02) after extracorporeal circulation. In contrast, none of the reductions in IL-6 and IL-10 by ε-aminocaproic acid achieved statistical significance. Both aprotinin and ε-aminocaproic acid decreased blood loss compared with saline, but there was no significant difference in the number of patients receiving blood products among the treatment groups.

Conclusions—These data suggest that aprotinin and ε-aminocaproic acid differ in their effects on the inflammatory response to extracorporeal circulation. Aprotinin but not ε-aminocaproic acid appears to attenuate the rise in the proinflammatory and anti-inflammatory cytokines IL-6 and IL-10. Further studies will be required to determine if these cytokine alterations translate to changes in clinical outcomes. (Circulation. 2001;104[suppl I]:I-265-I-269.)

Key Words: interleukins ■ antifibrinolytic agents ■ aprotinin ■ extracorporeal circulation ■ surgery

Patients undergoing cardiac surgery with extracorporeal circulation have a pronounced systemic inflammatory response, which leads to significant increases in both the proinflammatory cytokine interleukin (IL)-6 and the anti-inflammatory cytokine IL-10.1–6 Kawamura et al7 demonstrated that both IL-6 and IL-10 increase dramatically during extracorporeal circulation, peaking approximately 60 minutes after declamping of the aorta.

Aprotinin, a serine protease inhibitor, decreases the inflammatory response associated with extracorporeal circulation.1 Aprotinin inhibits plasmin-mediated fibrinolysis and many of the enzymatic intermediaries that contribute to the generalized extracorporeal circulation inflammatory response. Some but not all studies have shown that aprotinin effectively reduces the production of IL-6.7–11 The effect of aprotinin on IL-10, however, is not clear. Hill et al1 demonstrated that IL-10 levels were lower at 1 hour but higher at 24 hours after extracorporeal circulation in patients who received aprotinin (compared with saline placebo).

ε-Aminocaproic acid is commonly used in heart surgery to inhibit fibrinolysis and reduce blood loss after extracorporeal circulation. ε-Aminocaproic acid is a lysine analog that blocks binding of plasminogen and tissue plasminogen activator to fibrinogen, yet has no known effect on the systemic inflammation response.12 More specifically, the effects of ε-aminocaproic acid on IL-6 and IL-10 following extracorporeal circulation have not been reported. Our study is a randomized, double-blind, placebo-controlled study that was designed to evaluate the effect of both aprotinin and ε-aminocaproic acid on cytokines IL-6 and IL-10 and to test...
the hypothesis that inhibition of extracorporeal circulation–induced hyperfibrinolysis with aprotinin and 6-aminocaproic acid will decrease the production of the both the proinflammatory and anti-inflammatory cytokines after extracorporeal circulation.

Methods

Patient Selection
After institutional review board approval and written informed consent was given, 72 patients scheduled for elective, primary coronary artery bypass with extracorporeal circulation were enrolled in the study. Patients were randomly assigned to receive either (1) full-dose aprotinin (2×10⁶ KIU [load], 2×10⁸ KIU [pump prime], 5×10⁵ KIU/h [infusion]); (2) 6-aminocaproic acid (100 mg/kg [load], 5 g [pump prime], 30 mg/kg per hour [infusion]); or (3) saline (200 mL [load], 200 mL [pump prime], 50 mL/h [infusion]) in a double-blind fashion. Patients were excluded if they received corticosteroids, dipyridamole, or anticoagulants or had documented platelet or coagulation abnormalities or had treatment with thrombolytic therapy within 5 days of surgery. Other exclusion criteria included a creatinine level >2.0 mg/dL, a hemocrit fraction <30%, and a history of adverse reaction to aprotinin or 6-aminocaproic acid. Patients were not excluded from the study if they were receiving salicylates, nonsteroidal anti-inflammatory drugs, or heparin before surgery.

Technique of Operation
Preoperative medications, including β-blockers, nitrates, and antiarrhythmic agents, were continued until surgery. After sedation with 2 to 5 mg midazolam, intravenous access, a radial arterial cannula, and routine monitors were placed. After induction with 0.3 mg/kg etomidate, 5 to 10 μg/kg fentanyl, and 1 mg/kg rocuronium, a pulmonary arterial catheter was inserted through the right internal jugular vein. Anesthesia was maintained with inhaled isoflurane (0.2 mEq/kg per minute, was started at the beginning of rewarming during cardiopulmonary bypass and continued into the postoperative period for sedation. The following parameters were measured: heart rate, mean arterial blood pressure, peripheral arterial saturation, central venous pressure, mean pulmonary arterial pressure, pulmonary capillary wedge pressure, cardiac output (all measurements made in triplicate with the thermodilution technique), and continuous ST-segment analysis in ECG leads II and V₅. In the intensive care unit, sedation was provided as needed with 10 to 20 μg/kg per minute propofol and 2 mg IV morphine sulfate until arterial PₐO₂, arterial PₐCO₂, and white blood counts were measured at all data points. Medialastinal chest drainage was not reinfused. Packed red blood cells were transfused when the hemoglobin level fell below 8.0 g/dL, and platelet concentrates were given for platelet counts <70 000/μL in patients with clinical evidence of microvascular bleeding.

Interleukin Measurements
Arterial blood samples (5 mL) were collected at (1) baseline (before induction); (2) 10 minutes of extracorporeal circulation; (3) 30 minutes after extracorporeal circulation (after heparin reversal), (4) 3 hours after extracorporeal circulation; and (5) 18 hours after extracorporeal circulation. Blood was drawn from an arterial line directly into a polypropylene syringe and was transferred into a 3.2% sodium citrate–buffered Becton Dickinson brand Vacutainer. The concentrations of IL-6 and IL-10 in plasma were determined with a sandwich-type ELISA kit (R&D Systems). All standards and samples were assayed in duplicate.

Postoperative Physiological Variables
Hemoglobin, hematocrit, platelet counts, fibrinogen concentration, and white blood counts were measured at all data points. Mediastinal chest tube drainage and blood product administration was recorded for the first 24 hours after surgery. Postoperative physiological variables (body temperature, cardiac index, and systemic vascular resistance) were measured immediately after induction of anesthesia and 30 minutes after extracorporeal circulation. The duration of postoperative mechanical ventilation, the duration of inotropic support, and the length of the intensive care unit stay were recorded.

Statistical Analysis
A power analysis was performed to estimate the number of patients required for this study. On the basis of preliminary data, it was assumed that a change in interleukin levels of 15% would occur in the aprotinin group compared with the other groups. Assuming a 2-sided test with a probability of a type I error of 0.05 and a statistical power of 80%, it was calculated that each group would require at least 20 patients.

Statistical analysis was performed with SAS statistical software (SAS Institute). Differences in dichotomous variables between groups were evaluated by Fisher's exact test, and differences in continuous variables were analyzed by a 1-way ANOVA for normally distributed data and a nonparametric 1-way ANOVA when applicable. The behavior of each of the 3 treatment groups over time was analyzed by repeated-measures ANOVA. The treatment effect at a given time point was tested with the use of a Bonferroni adjustment for comparing means test. Differences were considered significant when probability values were less than or equal to 0.05. All values in the figures, tables, and text are expressed as mean±SD, unless otherwise stated.

Because length of operative procedure varied among patients, the exact timing of blood draws in relation to the onset of extracorporeal circulation had some variability. Because of the variability, interleukin levels were found to peak at different time points in the early postbypass period in different patients. To account for this difference, data from time points 30 minutes and 3 hours after extracorporeal circulation were combined to select a "peak" value for IL-6. For purposes of statistical analysis, data were analyzed by repeated-measures ANOVA by use of first, all 5 time points; then the use of (1) preinduction; (2) 10 minutes of extracorporeal circulation; (3) the peak value selected from 30 minutes and 3 hours after extracorporeal circulation; and (4) 18 hours after extracorporeal circulation.

Results
A total of 72 patients were randomly assigned to either the aprotinin (n=24), 6-aminocaproic acid (n=23), or saline (n=25) group. The patient and surgical demographic data are outlined in Tables 1 and 2, respectively. There were no significant differences between the patient and surgical demographic variables in any of the treatment groups except.
TABLE 1. **Patient Demographics**

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>EACA</th>
<th>Aprotinin</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>25</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td><strong>General</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>62±7</td>
<td>63±8</td>
<td>64±9</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>2.1±0.2</td>
<td>2.1±0.2</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>55.3±15</td>
<td>58.2±15</td>
<td>55.5±13</td>
</tr>
<tr>
<td><strong>Medical, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H/o myocardial infarction</td>
<td>16±55</td>
<td>11±52</td>
<td>11±46</td>
</tr>
<tr>
<td>H/o congestive heart failure</td>
<td>7±24</td>
<td>4±17</td>
<td>6±25</td>
</tr>
<tr>
<td>Hypertension</td>
<td>19±66</td>
<td>16±76</td>
<td>19±79</td>
</tr>
<tr>
<td>Diabetes</td>
<td>14±48</td>
<td>10±48</td>
<td>7±29</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>2±7</td>
<td>4±19</td>
<td>4±17</td>
</tr>
<tr>
<td>H/o smoking</td>
<td>24±83</td>
<td>14±67</td>
<td>17±71</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>17±59</td>
<td>17±81</td>
<td>17±71</td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin therapy</td>
<td>23±79</td>
<td>19±90</td>
<td>21±88</td>
</tr>
<tr>
<td>Intraoperative heparin</td>
<td>7±24</td>
<td>5±24</td>
<td>5±21</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>24±83</td>
<td>17±81</td>
<td>20±83</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>20±69</td>
<td>14±67</td>
<td>19±79</td>
</tr>
</tbody>
</table>

Values are mean±SD or n (%).

EACA indicates ε-aminocaproic acid; BSA, body surface area; and H/o, history of.

The influence of extracorporeal circulation on IL-10 for 4-hour blood loss. Patients in the aprotinin and ε-aminocaproic acid groups had significantly less blood loss compared with the saline group (P<0.05). There was no significant difference in blood loss noted between patients in the aprotinin and ε-aminocaproic acid groups.

The effect of extracorporeal circulation on IL-10 is illustrated in the Figure. There were no significant differences in the baseline IL-10 between groups. Administration of aprotinin resulted in a significant overall treatment effect compared with saline (P=0.02), whereas ε-aminocaproic acid did not (P=0.51). In addition, no significant differences were noted between patients in the aprotinin and ε-aminocaproic acid groups (P=0.10). IL-10 levels at 18 hours after extracorporeal circulation were significantly elevated compared with preinduction (P<0.05) yet did not differ between treatment groups.

Compared with saline, peak IL-6 was significantly lower in the aprotinin (P=0.024) but not the ε-aminocaproic acid (P=0.46) group in the early postbypass period (30 minutes to 3 hours). IL-6 levels at 18 hours after extracorporeal circulation were also significantly increased in all treatment groups compared with preinduction levels (P<0.01).

The effect of aprotinin and ε-aminocaproic acid on IL-10, extracorporeal circulation time, and blood transfusions on peak IL-6 and IL-10 were analyzed overall and within each treatment group. We found no correlation between peak IL-6 or IL-10 and aortic cross-clamp time or total extracorporeal circulation times. When analyzed by treatment group, there was a weak correlation between peak IL-6 and aortic clamp (r=0.550) and total extracorporeal circulation (r=0.555) times in the ε-aminocaproic acid group only. There were no significant differences in the overall and peak IL-6 and IL-10 levels between those receiving blood products (before drawing these samples) and those who did not receive blood products. The percentage of subjects receiving blood products during surgery and the early after the bypass period did not differ between treatment groups, nor did their peak levels of IL-6 or IL-10 between each group (Table 2).

Table 3 lists postoperative physiological and clinical milestones for each of the 3 treatment groups. The complete blood counts were not significantly different, with the exception of the white blood cell count between patients in the ε-aminocaproic acid and saline groups (P<0.05).

**Discussion**

Although multiple studies have demonstrated that heart surgery with extracorporeal circulation significantly increases proinflammatory and anti-inflammatory cytokines, the effect of aprotinin and ε-aminocaproic acid on the cytokine synthesis inhibitory factor (CSIF) IL-10 remains unclear. The findings from this study indicate that high-dose aprotinin significantly attenuates increases in IL-10 seen in patients after extracorporeal circulation. The decreased IL-10 response in the aprotinin group was also associated with a reduction in peak IL-6 levels. A significant effect of ε-aminocaproic acid on the IL-10 and IL-6 response was not identified.

The significant increase and time course of peak IL-10 levels in our study was consistent with previously reported data in patients undergoing cardiac surgery with extracorporeal circulation. The effect of high-dose aprotinin on the IL-10 response after extracorporeal circulation is less clear. Hill and colleagues showed a minor reduction in IL-10 levels with aprotinin in the period immediately after extracorporeal circulation, but paradoxically reported a significant increase in IL-10 levels in the aprotinin group 24 hours after surgery (compared with a saline control group). Some investigators have suggested that the anti-inflammatory effects of aprotinin are similar to steroids, yet only steroids have consistently increased IL-10 levels after extracorporeal circulation in well-controlled trials. These data suggest that the mechanism by which aprotinin and steroids influence the balance of proinflammatory and anti-inflammatory cytokines may differ.
TABLE 2. Surgical Demographics

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Saline</th>
<th>EACA</th>
<th>Aprotinin</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of distal anastomoses</td>
<td>3.3±1.0</td>
<td>3.4±0.6</td>
<td>3.3±0.9</td>
</tr>
<tr>
<td>Aortic cross-clamp time, min</td>
<td>74±23</td>
<td>72±19</td>
<td>76±26</td>
</tr>
<tr>
<td>ECC time, min</td>
<td>123±36</td>
<td>128±39</td>
<td>126±33</td>
</tr>
</tbody>
</table>

Anticoagulation

| Heparin, units×100              | 477±141     | 438±101     | 464±102     |
| Protamine, mg                   | 338±110     | 317±98      | 332±90      |

Blood loss/transfusions

| 4-h blood loss, median (25th, 75th) | 372 (200, 405) | 192* (100, 366) | 100* (95, 215) |
| Excessive early blood loss, n (%)  | 5 (25)       | 3 (13)       | 0 (0)        |
| Transfused intraoperatively and early post-ECC, n (%) | 6 (24) | 6 (26) | 5 (21) |
| Transfused before discharge, n (%) | 13 (45) | 5 (24) | 7 (29) |

Values are mean±SD unless otherwise indicated. EACA indicates ε-aminocaproic acid; ECC, extracorporeal circulation; and excessive blood loss, >600 mL of mediastinal chest tube drainage in the first 4 hours after placement.

*P<0.05 compared with saline group.

The response of IL-6 levels to the stimulus of extracorporeal circulation seen in this study was qualitatively similar to that observed for IL-10. A significant reduction in peak IL-6 levels after extracorporeal circulation was identified with aprotinin but not ε-aminocaproic acid. These findings are consistent with studies reported elsewhere, although other investigators have failed to identify any modulating effect using lower doses of this drug.

Increases in IL-10 after heart surgery are believed to represent an endogenous reaction aimed at suppressing the effect of the proinflammatory cytokine response. The clinical relevance of elevated anti-inflammatory cytokines, such as IL-10, after extracorporeal circulation surgery is not clear. Multiple studies have demonstrated that steroids increase IL-10 and decrease the proinflammatory cytokines (IL-6 and IL-8). Patients receiving steroids achieve higher IL-10 levels and show signs of improved respiratory and hemodynamic function after extracorporeal circulation surgery. In contrast, elevations in IL-10 have also been associated with an increased mortality in patient with febrile disease and morbidity (infection and so forth) in selected groups of patient undergoing extracorporeal circulation.

The reasons for the decrease in the IL-10 response with aprotinin found in the present study are not known. If IL-10 levels represent a response to the proinflammatory cytokines (such as IL-6) that are invoked by extracorporeal circulation, then reduction in proinflammatory molecules by aprotinin may be the primary event. Alternatively, aprotinin may directly affect the production and/or release of IL-10 by monocytes and T-cells. Additional studies will be required to identify the mechanism of this observation. ε-Aminocaproic acid did not significantly reduce the responses of either IL-6 or IL-10 compared with saline controls. This lysine analogue is generally believed to have a more limited effect on the inflammatory response, with its major putative effect directed toward inhibition of clot-bound plasmin activity.

Study Limitations

Like all clinical investigations, there are significant limitations to the conduct of the study and interpretation of the data. Blood samples are collected at time points determined by the progress of the operation (in relation to extracorporeal circulation, protamine administration, or temperature) but not at fixed times after skin incision because of variability in the length of the surgical procedures. Studies that use additional time points have shown that IL-6 levels sharply peak then rapidly fall over the first 6 hours after extracorporeal circulation. Furthermore, patients in the present study were subjected on average to a longer period of extracorporeal circulation than that reported in other studies. Because longer duration of extracorporeal support appears to represent a...
greater stimulus to the inflammatory response, this may in part explain why other investigators have not identified an effect of aprotinin on IL-10 levels.

The use of blood products, especially platelet concentrates, can increase circulating IL-6 levels. The subset of our patient population receiving blood products before the peak of IL-6 and IL-10 was small (≈25%), and subgroup analysis of patients not receiving blood products did not alter our findings. Finally, the ultimate effect of modulation of the inflammatory response on clinical outcomes such as infection, respiratory failure, or survival cannot be determined in a study of this size.

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