Anti-Inflammatory Effect of Aprotinin: A Meta-Analysis

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Abstract: It is important to define the extent, and any limitations, of potential anti-inflammatory regimens used in cardiac surgery to guide the rational combination of drugs to suppress the systemic inflammatory response. Aprotinin (Trasylol) is an anti-fibrinolytic agent with reported anti-inflammatory properties. In this study, we investigated the published data on aprotinin’s effect on acute phase protein and cytokine levels in cardiac surgery patients. Randomized placebo-controlled trials of aprotinin published between 1985 and 2007, in adult cardiac surgery using cardiopulmonary bypass, reporting tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-8, and IL-10 levels were included for review. Two independent reviewers graded each paper and collected information on inflammatory markers. RevMan 4.3 statistical software was used to calculate and plot the weighted mean difference between placebo and aprotinin groups. Thirteen studies met the review criteria. None of the inflammatory markers were reduced by high-dose aprotinin treatment. Low-dose aprotinin significantly reduced IL-10 levels after protamine administration (−41.3 pg/mL; 95% CI: −59.5, −23.1), but this result was gone by the first post-operative day. These meta-analyses showed no significant effect of aprotinin on acute phase proteins or systemic cytokine markers of inflammation during clinical adult cardiac surgery using cardiopulmonary bypass. While recognizing that other host defense systems, such as coagulation and complement, contribute to the overall systemic inflammatory response, the evidence presented here does not support the clinical use of aprotinin as an anti-inflammatory agent on its own. Keywords: meta-analysis, aprotinin, surgery, inflammation.

The systemic inflammatory response is a homeostatic response of the body to the combined insults of surgery and contact of blood with the foreign surface of the bypass circuit. It is characterized by activation of complement, coagulation, fibrinolytic, and kallikrein cascades, activation of neutrophils with degranulation and protease enzyme release, oxygen radical production, and the synthesis of various cytokines from mononuclear cells (1–3). If unchecked, these activated defense systems are associated with increased risk of organ injury and death (4).

Aprotinin (Trasylol; Bayer Pharmaceuticals, West Haven, CT) has been marketed in cardiac surgery as an anti-fibrinolytic agent to reduce bleeding. In addition, over the past 10 years, Bayer Pharmaceuticals has also encouraged cardiac care teams to use aprotinin as an anti-inflammatory agent. The clinical evidence for anti-inflammatory properties of aprotinin is mixed, with the most notable benefits reported for stroke in meta-analyses (5) and a Cochrane database review (6). However, aprotinin may incur significantly increased risk of death and renal injury compared with other anti-fibrinolytic agents (7,8). The safety issue remains a controversial and hotly debated topic, which is not the purpose of this study (9).

The purpose of this meta-analysis is to clarify whether aprotinin possesses anti-inflammatory effects on acute phase protein and inflammatory cytokine generation supported by published clinical data. Most of the potential anti-inflammatory mechanisms for aprotinin have been identified in animal studies or ex vivo models of human vascular cell activation (10–16). Despite a large number of trials evaluating clinical endpoints (bleeding and transfusion), there has been limited clinical evidence supporting the anti-inflammatory properties of aprotinin. Therefore, we conducted a series of meta-analyses on the effect of aprotinin on acute phase proteins and systemic cytokine markers of inflammation reported in randomized control trials: tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-8, and IL-10.
MATERIALS AND METHODS

Study Selection

We conducted a meta-analysis of randomized clinical trials (RCTs) of use of aprotinin in adult cardiac surgery using cardiopulmonary bypass including cases of coronary artery bypass surgery. Primary and re-operations for coronary artery bypass graft (CABG), valve, and concomitant valve/CABG surgeries were included in this analysis. OVID/MEDLINE was used to identify published RCTs from 1985 through 2007. Key words used to search included the following: Trasylol or aprotinin, inflammation or cytokine, cardiopulmonary bypass or cardiac surgery. The search yielded 49 published human RCTs (Figure 1). The search was further limited to the English language (45 studies) and adults (38 studies). Among the 25 studies remaining, reported outcomes for TNF-α (8 studies), IL-6 (17 studies), IL-8 (12 studies), and IL-10 (8 studies) were found. After careful review of each trial, trials were excluded for not reporting the results of the marker of interest or reporting the median and interquartile range instead of the mean and SD or SEM. Any study not reporting mean and SD or SE was not included in the summary statistic calculation of the weighted mean difference. We requested the authors of these studies to send us the mean and SD of the studies; three replied with tables (17,18) or raw data to be analyzed (19). We are grateful to those authors for the supplemental data and cooperation.

Four additional trials were excluded for reporting cytokine levels outside detectable or clinical ranges (<5 pg/mL, >50 ng/mL) (20–23). These exclusions resulted in 13

![Figure 1. RCTs reporting inflammatory markers.](image-url)
Table 1. Characteristics of the included trials.

<table>
<thead>
<tr>
<th>Principal</th>
<th>Year</th>
<th>N_total</th>
<th>N_Treatment</th>
<th>N_Control</th>
<th>Aprotinin Dose</th>
<th>Surgical Procedure</th>
<th>Jadad Score (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defraigne</td>
<td>2000</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>2 million KIU</td>
<td>CABG</td>
<td>3</td>
</tr>
<tr>
<td>Diego</td>
<td>1997</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>2 million KIU and 1 million KIU</td>
<td>CABG</td>
<td>2</td>
</tr>
<tr>
<td>Goudeau</td>
<td>2007</td>
<td>27</td>
<td>13</td>
<td>14</td>
<td>2 million KIU</td>
<td>CABG, valve</td>
<td>2</td>
</tr>
<tr>
<td>Greilich</td>
<td>2001</td>
<td>49</td>
<td>24</td>
<td>25</td>
<td>2 million KIU</td>
<td>CABG</td>
<td>3</td>
</tr>
<tr>
<td>Greilich</td>
<td>2003</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>2 million KIU</td>
<td>CABG</td>
<td>1</td>
</tr>
<tr>
<td>Harig</td>
<td>1995</td>
<td>16</td>
<td>8</td>
<td>8</td>
<td>1 million KIU</td>
<td>CABG</td>
<td>1</td>
</tr>
<tr>
<td>Hill</td>
<td>1998</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>2 million KIU</td>
<td>CABG</td>
<td>2</td>
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<tr>
<td>Kaminishi</td>
<td>2004</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>1 million KIU</td>
<td>CABG</td>
<td>1</td>
</tr>
<tr>
<td>Schmartz</td>
<td>2003</td>
<td>79</td>
<td>41</td>
<td>38</td>
<td>2 million KIU and 1 million KIU</td>
<td>CABG</td>
<td>4</td>
</tr>
<tr>
<td>Tassani</td>
<td>2000</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>2 million KIU</td>
<td>CABG</td>
<td>3</td>
</tr>
<tr>
<td>Turkaz</td>
<td>2001</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>2 million KIU</td>
<td>CABG</td>
<td>2</td>
</tr>
<tr>
<td>Wei</td>
<td>2001</td>
<td>21</td>
<td>12</td>
<td>9</td>
<td>280-mg pump</td>
<td>CABG</td>
<td>2</td>
</tr>
</tbody>
</table>

KIU, kallikrein inhibitor units (pg/mL).

trials to review (Table 1): TNF-α (4 articles) (19,24–26), IL-6 (10 articles) (17–19,24,26–31), IL-8 (6 articles) (17–19,24,26,31), and IL-10 (5 articles) (18,19,31–33).

We abstracted data at two commonly reported time points: after protamine administration and on post-operative day 1 (~12–24 hours after surgery). We followed the appropriate methods for conducting a meta-analysis as stipulated in the CONSORT statement (34). Two independent reviewers (JB and AT) selected trials for information outcomes and recorded data on spreadsheets. Jadad criteria were assessed by the two reviewers (35). If disagreements were not resolved by a second review, they were resolved by a third reviewer (RK).

To control for the dosing effect of aprotinin, we stratified our analysis by high- and low-dose aprotinin (as suggested by the manufacturer). High-dose, also referred to as Hammersmith or full-dose, aprotinin consisted of a 2 million KIU (kallikrein inhibiting units) intravenous loading dose, 2 million KIU pump-priming dose, and 0.5 million KIU/h intravenous maintenance dose. Low-dose (also referred to as half-dose) aprotinin consisted of a 1 million KIU intravenous loading dose, 1 million KIU pump-priming dose, and 0.25 million KIU/h intravenous maintenance dose. Cardiopulmonary bypass pump-only aprotinin was included in our low-dose analysis. The overall effect (irrespective of dose) was calculated.

Statistical Analysis

The weighted mean difference (WMD) and 95% confidence intervals (CIs) were calculated to compare the mean difference in inflammatory marker levels using the Cochrane Collaborative software, RevMan 4.3. In addition, we calculated the I² to evaluate the percentage of heterogeneity among all the trials incorporated in the summary estimate (36). To control for heterogeneity, we used random effects modeling.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

RESULTS

Of the 13 studies reporting inflammatory markers of interest, 4 studies reported on TNF-α, 10 on IL-6, 6 on IL-8, and 5 on IL-10 (Figure 1). Neither high- nor low-dose aprotinin achieved statistically significant inhibition of perioperative TNF-α, IL-6, or IL-8 levels (Figures 2–4). Only low-dose aprotinin significantly reduced IL-10 levels after protamine administration (−41.3 pg/mL; 95% CI: −59.5, −23.1) but not on postoperative day 1 (Figure 5).

DISCUSSION

We identified 25 randomized controlled trials reporting TNF-α, IL-6, IL-8, and IL-10 outcomes after protamine administration and on post-operative day 1; only 13 of those trials reported or sent data for use in the meta-analyses. Significant suppression was noted only for IL-10 using low-dose aprotinin at the protamine reversal time point, implying a net pro-inflammatory effect at this time point. Hence, we found no evidence for statistically significant anti-inflammatory effects on any of the acute phase proteins (TNF-α, IL-6), inflammatory cytokine (IL-8), or anti-inflammatory cytokine (IL-10) studied.

Other individual randomized trials have studied alternative inflammatory endpoints. One trial reported a significant reduction in C-reactive protein (CRP) (28), whereas three trials showed no reduction in complement 3 and 4 (C3 and C4) (37–39).
Although the evidence base for aprotinin is therefore limited, the evidence is even more sparse for other anti-fibrinolytics (the synthetic lysine analogs e-amino caproic acid and tranexamic acid). Greilich et al. (4,17,32) reported three times that IL-6 and IL-10 were not reduced by e-amino caproic acid. However, Greilich et al. (17) reported a benefit in IL-8 reduction. In one further trial, Casati et al. (40) reported a significant reduction in IL-6 by tranexamic acid.

It is telling that this first meta-analysis of the anti-inflammatory effect of aprotinin should take place after the drug has been suspended from the market. Aprotinin is a cautionary tale for when clinicians and marketing get ahead of the science. The purported anti-inflammatory benefits were marketed ahead of the scientific evidence base and ahead of more important clinical safety considerations, such as weight-based dosing (41,42). It is imperative that future anti-inflammatory interventions are based on the consensus that randomized trials should measure appropriate inflammatory markers and clinical endpoints with the statistical power to establish an anti-inflammatory evidence base (43).

The challenge for cardiac care teams in managing the systemic inflammatory response lies in achieving simultaneous blockade of the many arms contributing to this multi-system disorder. Every major host defensive system of the body is activated, from complement to coagulation and fibrinolysis, leukocyte and endothelial activation,
proteolytic mediator release, free radical generation, and inflammatory cytokine release (2). This study examined only one aspect of the inflammatory response, acute phase protein and cytokine release, with one drug intervention, yet studies of this nature will become critical if we are to achieve evidence-based intervention strategies using combinations of drugs and other anti-inflammatory regimens (43,44). Thus, although we found no evidence for anti-inflammatory effects against acute phase or cytokine markers, it remains conceivable that aprotinin may successfully target leukocyte activation, diapedesis, or extravascular fluid shifts (10,11,15,16,25,33,45–47). Regardless of the safety issues surrounding this particular compound, aprotinin can serve as a template for other serine protease inhibitors.
inhibitors in the drug pipeline, which remain attractive because of their targeting of multiple pathways in the body’s response to cardiopulmonary bypass. The challenge is to identify combinatorial drug regimens to complement the inhibitory profile of aprotinin (e.g., possible combination with steroids or anti-complement agents).

Our analysis has some limitations. Because of publication bias, we may have underestimated the lack of benefit, assuming that there may be unpublished studies that showed no difference. The largest studies centered around the null, whereas the smaller studies showed mixed results. The potential is not excluded for aprotinin to attenuate the inflammatory response in high-risk subgroups, an outcome that would not be detected in this analysis, because some of the studies were small and included low-risk patients. In addition, there were likely covariates at work in all of the studies that may have influenced the inflammatory response.

We discovered that aprotinin failed to show any benefit in reducing inflammation peri-operatively. The weight of evidence does not support the use of aprotinin as an anti-inflammatory agent on its own.

Figure 4. Meta-analysis of IL-8. Individual RCTs are listed in order by year of publication. The size of each square denotes the weight of each trial’s weighted mean difference in calculating the summary estimate for the overall effect on IL-8. The diamond represents the summary estimate for the combined weighted mean difference at the center; opposing points of the diamond represent the 95% CIs. Three diamonds in each section represent high, low, and overall effect. A, Meta-analysis for IL-8 directly after protamine administration. B, Meta-analysis plot for IL-8 on post-operative day 1.
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