Original Article

The Antiinflammatory Effects of Aprotinin in Patients Undergoing Cardiac Surgery with Cardiopulmonary Bypass

Alfred H. Stammers, MSA, CCP[+]; Suzanne Huffman, BS, CCP[+]; Anselmo Alonso, MD[*]; Lance W. Fristoe, BS, CCP[+]; Gary Hill, MD[^]; Dana Casebeer, PharmD[**]; Robert P. Diego, BS[++]; Zuorui Song, MD[+]

Division of Clinical Perfusion Education[+], Departments of Cardiothoracic Surgery[*], Anesthesia[^], and Pharmacology[**], and College of Medicine[++], University of Nebraska Medical Center, Omaha, Nebraska

Keywords: cardiopulmonary bypass, inflammation, coagulation, aprotinin, serine protease inhibition

Presented at the American Society of Extra-Corporeal Technology 35th International Conference, April 3-6, 1997, Phoenix, Arizona, by Alfred Stammers, winner of the 1997 Fellowship Award.

ABSTRACT

Aprotinin has been shown to effectively attenuate cardiopulmonary bypass (CPB) induced coagulopathies. Because aprotinin is a serine protease inhibitor, it may exert additional properties that reduce the risks associated with extracorporeal flow. The purpose of this study was to prospectively evaluate the antiinflammatory effects of aprotinin with specific emphasis on pulmonary function.

After Institutional Review Board approval, 20 patients undergoing first time coronary artery bypass grafting were randomly assigned to receive either a full dose regimen of aprotinin (APR, n=8), or volumetric equal control (CTR, n=12). Biological markers of inflammation and coagulation were measured at 3 time periods: immediately prior to drug administration, at chest closure, and at 24 hours post cardiotomy, and included total complement, polymorphonuclear neutrophil (PMN) elastase, Factor XII, protein C, protein S, fibrin split products (FSP), D-dimers. Pulmonary function was assessed throughout intensive care unit (ICU) stay.

There were no differences observed between groups in either preoperative, surgical, anesthesia or perfusion parameters. Twenty-four hour chest tube drainage in the APR group was significantly less than that observed in CTR patients (435.1±169.6 vs. 944.0±585.1, p<.02). Patients receiving aprotinin received significantly lower transfusions of red blood cells, platelets, and fresh frozen plasma. Upon entry into the ICU the CTR group had significantly higher mean airway pressures (8.3±1.5 vs. 10.8±2.9 cm H2O, p<.03), higher $PaCO_2$ levels (37.1±4.8 vs. 43.3±7.1 mmHg, p<.04), and higher FIO_2 settings (0.63±0.18 vs. 0.75±0.20, p=.16). Postoperative FSP and D-dimers were significantly lower in the APR treated patients.

In conclusion, the use of aprotinin resulted in significant improvements to postoperative patient outcomes as assessed by transfusion requirements, blood loss, coagulation markers and pulmonary function.

Address communications to:
Alfred H. Stammers, MSA, CCP
Division of Clinical Perfusion Education
University of Nebraska Medical Center
600 South 42nd Street
Omaha, NE 68198-5155

INTRODUCTION

The process of extracorporeal circulation results in dramatic changes to both biochemical and cellular elements of blood, with these derangements upsetting the delicate hemostatic balance. In addition to the effects on coagulation, various other protein mediated systems are activated which contribute towards the morbidity associated with extracorporeal flow. Activation of complement has been shown to release powerful anaphylatoxins which induce an inflammatory type whole body reaction that has been well described (1,2). Other proinflammatory events implicated in this phenomena include neutrophil activation (3), the production of reactive oxygen species (4), and cytokines such as interleukin-8 and tissue necrosis factor-α (5-7).

Methods to reduce these negative effects have included modification of extracorporeal circuitry through heparin-coating techniques (8), the use of leukocyte depleting filters (9), and the application of various pharmacological agents (5,10-12), all of which are purported to improve the biocompatible nature of cardiopulmonary bypass (CPB). Of the pharmacological agents, aprotinin seems to offer the most promise in modulating many of the injurious mechanisms that disrupt homeostasis in patients undergoing cardiac surgery with CPB (13-17).

Aprotinin is a serine protease inhibitor that inhibits kallikrein, plasmin, and trypsin and has been shown to be effective in reducing postoperative bleeding and homologous blood transfusion following CPB (18). Some investigators have shown that the antiproteolytic activity of aprotinin may exert antiinflammatory properties (19,20), while others have not supported this claim (21). The purpose of the present study was to examine the antiinflammatory effects of aprotinin in patients undergoing coronary artery bypass grafting (CABG) with CPB.

MATERIALS AND METHODS

Study Protocol: The study design included a randomized, prospective, double-blind, placebo controlled study of aprotinin administration. After obtaining Institutional Review Board approval, and informed consent, 20 adult patients undergoing elective coronary artery bypass grafting (CABG) were entered into this study. Patients were randomized to either high dose aprotinin (APR) or control (CTR) groups. The treatment group received aprotinin according to the manufacturer's instructions for use. Following a test dose of 1 ml, a loading dose of 2 X 10^6 (280 mg) KIU (kallikrein inactivator units) was administered intravenously (IV) immediately following the induction of anesthesia. 2 X 10^6 KIU were placed in the priming volume of the extracorporeal circuit, and patients received a constant infusion of 5 X 10^5 KIU (70 mg) IV hourly until chest closure. The CTR patients received an equal volume of saline administered in the same manner. All drugs were drawn up immediately prior to use by a pharmacist (DC) and placed in a 500 ml evacuated glass bottle which was labeled with the patient's name, registration number and date. No other clinician knew of the treatment received by the patient.

All patients ranged in age between 19 and 80 years with either gender accepted. Specific exclusion criteria included any of the following: semilunar valvular stenosis with aortic valve area of 0.6 cm^2 or less, atrioventricular or semilunar valve regurgitation, preoperative coagulation disorders as assessed by a prothrombin time (PT) greater than 1.5 times normal, preoperative hematocrit less than 33%, or patient weight less than 50 kg.

All patients received identical surgical, anesthesia and postoperative care and were operated on by one surgeon (AA). Immediately prior to surgery a radial artery catheter, a right internal jugular vein pulmonary artery catheter, and large bore IV lines were placed. Standard anesthesia consisting of fentanyl (75-100 ug/kg) and pancuronium (0.1-0.2 mg/kg) was used. The perfusion circuit consisted of a centrifugal pump[a], membrane oxygenator[b], collapsible venous reservoir[c], arterial line filter[d], and filtered cardiotomy reservoir[e]. The prime solution consisted of approximately 1800 ml of balanced electrolyte solution to which was added 100 ml 25% albumin, 100 ml 25% mannitol, and 10,000 IU of bovine lung heparin. During CPB arterial pressures were maintained between 60-80 mmHg, with flow rates adjusted between 2 and 2.4 L/min/m^2. Mild hypothermia (32°C) was used and blood gas homeostasis was maintained according to alpha-stat physiology. Left ventricular venting was accomplished by placing a 20 F catheter in the right superior pulmonary vein and advancing it into the left ventricle. Myocardial protection was achieved with both antegrade and retrograde blood cardioplegia (8:1 blood to crystalloid ratio) being used, with potassium concentrations adjusted for arrest (24-28 mEq/L), and for maintenance (8-10 mEq/L) administered at the conclusion of each distal graft. A terminal dose of warm (37°C) cardioplegia was administered to all patients over a 10 to 20 minute period prior to removal of the aortic cross clamp. Anticoagulation was obtained by the administration of bovine lung heparin (300 IU/kg), and kaolin activated clotting times (ACT) were maintained at 500 sec or greater in both groups by the administration of additional heparin when necessary. Following CPB, protamine was administered in a ratio of 1.3 mg for every 100 IU of total heparin administration, and confirmed by the return of ACT to baseline values.

Patient parameters consisted of demographic, operative, postoperative and laboratory measures. Patients were monitored continuously throughout the operative procedure and until discharge from the intensive care unit (ICU), with all transfusion requirements recorded. Patients were transfused with packed red blood cells (PRBC) when the hemoglobin level fell below 7 gm/dL, in patients less than 80 years of age, and 8 gm/dL, in

a BP-80, Medtronic Biomedicus, Inc., Eden Prairie, MN
b Monolyth Oxygenator, Sorin Biomedical, Irvine, CA
c BMR 1900, Baxter Healthcare Corp., Irvine, CA
d ALF 1040, Baxter Healthcare Corp., Irvine, CA
e Card 3IF, Sorin Biomedical, Irvine, CA

patients over 80 years of age. Patients were also transfused with PRBC when hemodynamic instability due to anemia was suspected. Patients were transfused with coagulation factors in the form of fresh frozen plasma (FFP), platelets (PLT), and cryoprecipitate (CRYO) only when bleeding was uncontrolled, assessed either by direct observation in the operating room or via chest tube drainage (two consecutive hours of chest tube output exceeding 300 ml/hr). Criteria for transfusion was as follows: FFP when PTs were greater than 1.5 times normal, PLT when platelet counts fell below 100,000/uL, and CRYO when fibrinogen levels were less than 100 mg/dL.

Laboratory Assessment: In addition to standard laboratory assessment, preoperative and postoperative coagulation determinations of PT, activated partial thromboplastin time (aPTT), and platelet count were drawn in the immediate preoperative and postoperative periods. Additional coagulation and inflammatory markers were drawn at three distinct times: preoperatively (post-induction and prior to surgical incision), postoperatively (at chest closure), and on the first postoperative day (24 hours post chest closure). During each of these times the following parameters were measured: total complement (CH_{100}), fibrin(ogen) split products (FSP), D-dimers, protein C and protein S, factor XII and PMN elastase. Samples for FSP, D-dimers, protein C and protein S were immediately performed by separating plasma from whole blood at 2500 G for 10 minutes, supernating the plasma, and performing the assays as described below. After separation an additional aliquot was immediately frozen and stored at -70°C for polymorphonuclear neutrophil (PMN) elastase determination.

Fibrin(ogen) split products were measured using latex particles coated with antibodies to human fibrinogen fragments D and E, with results expressed in mg/dL. D-dimers were assayed utilizing mouse anti-human D-dimer monoclonal antibodies. The test for D-dimers detects only cross linked fibrin degradation products and did not detect FSP, and results are expressed in ng/ml. Factor XII activity was determined by the degree of correction of the aPTT obtained when dilutions of the test plasma are added to severely deficient substrate plasma, with the results compared to the degree of corrections obtained when dilutions of normal plasma with a known factor activity are added to the same severely factor deficient substrate plasma. Protein C activity was measured using a commercially available assay kit[f] (PROTAC™), while protein S activity was measured using the ASSERA®PLATE kit[g]. Factor XII, protein C and protein S results are expressed in % activity. PMN elastase was measured with an enzyme immunoassay kit from Merck[h] containing polymorphonuclear leukocytes in complex with alpha-1 proteinase inhibitor, and the results are expressed in ug/L.

Statistics: Statistical analysis was performed by loading all data onto a personal computer in a spread sheet format. Parametric data were analyzed using a one-way analysis of variance (ANOVA). Additional multiple comparison tests (Scheffe's Test) were performed when significant ($p<.05$) "F" ratios achieved. A Fisher's exact test was used to compare the FSP and D-dimer data. Non-parametric data was analyzed using a Mann-Whitney U test. Values of p less than or equal to 0.05 were considered to be statistically significant. All data are reported as mean ± standard deviation of the mean, unless otherwise stated.

f American Diagnostica, New York, NY
g Diagnostica Stago, American Bioproducts, Parsippany, NJ
h 12589 PMN Elastase IMAC, Merck, Darmstadt, Germany

Table 1: Patient demographic data

Parameter	Aprotinin Group	Control Group	p Value
Number	8	12	
Age (years)	66.3 ± 5.8	63.9 ± 9.2	NS
Gender (male/female)	6/2	10/2	NS
Weight (kg)	85.3 ± 9.9	87.7 ± 19.4	NS
Height (cm)	172.7 ± 9.4	174.2 ± 8.7	NS
BSA (m^2)	2.00 ± 0.2	2.03 ± 0.2	NS
Preop ASA Use (%)	75	58	NS
Preop Eject Fraction (%)	54 ± 13	57 ± 13	NS

Legend: ASA = Acetylsalicylic acid; BSA = Body surface area.
All data are mean ± SDEV.

Table 2: Perioperative data

Parameter	Aprotinin Group	Control Group	p Value
CABG (graft #)	3.8 ± 0.9	4.1 ± 1.4	NS
Internal Thoracic Artery Usage (%)	100	75	NS
CPB Time (min)	120.4 ± 32.6	114.3 ± 52.2	NS
Cross Clamp Time (min)	85.3 ± 27.1	82.7 ± 34.3	NS
Prime Volume (ml)	2065 ± 115	2100 ± 213	NS
Total Heparin Use (U)	25912 ± 3014	29375 ± 9795	NS
Total Protamine Use (mg)	372.5 ± 170.0	429.2 ± 181.6	NS
Operative Urine Output (ml)	1219 ± 659	1602 ± 570	NS

Legend: CABG = Coronary artery bypass graft; CPB = Cardiopulmonary bypass.
All data are mean ± SDEV.

RESULTS

There were no differences between either group in any demographic parameters (Table 1) which included cardiac risk factors and preoperative medication use. Both groups received equal operative care and there were no differences in CPB or cross clamp times, nor the use of the internal thoracic artery (Table 2). Patients

in the treatment group had lower total operative urine output although statistical significance was not achieved.

Transfusion data is shown in Table 3 with patients in the APR group receiving significantly fewer transfusions than CTR patients; both in the operating room and for total time in the hospital (Figures 1a and 1b). The average PRBC transfusion rate was three times greater in the CTR group than APR, while both FFP and PLT transfusions were approximately two times greater in CTR patients.

There were no significant differences between groups at any time period in the following perioperative laboratory tests: hemoglobin, PT, white blood cell count, or platelet count (Table 4). The aPTT in the immediate postoperative period was significantly higher in the APR group compared to CTR. The ACT, completed just prior to the aPTT test, displayed a reverse trend with the highest value (141.6±67.5 sec.) found in the CTR patients (p=ns).

Factor XII values declined more in the APR group, falling to 58% of preoperative values at the end of chest closure, while the CTR declined to only 84% (p=ns). Both groups returned to baseline values at the end of the first postoperative day. There were no significant differences between groups found in either protein C or protein S activity, which reflected a dilutional decline during the operative period. Neither parameter, however, returned to baseline values at the end of the first postoperative day, reaching only 74% of preoperative levels.

FSP and D-dimers were elevated during both postoperative times in each group (Figures 2 and 3). Despite a trend towards increased fibrinolytic activity in the control group, statistical significance was only achieved during the first postoperative time period in D-dimer measurements (Figure 3). Total complement (CH_{100}) declined in consecutive sample points and returned to near baseline values in the CTR group. In the APR group, however, CH_{100} was approximately 35% below the baseline value (p=ns) at 24 hours post chest closure. There were no significant differences in CH_{100} seen between groups at any time.

PMN elastase levels increased in both groups during the operation, and remained elevated on the first postoperative day (Figure 4). The APR group had a significantly lower increase in PMN elastase than CTR at chest closure, with the levels returning to near baseline values at the 24 hours postoperative time point. The CTR had elevated PMN elastase levels at both postoperative time periods.

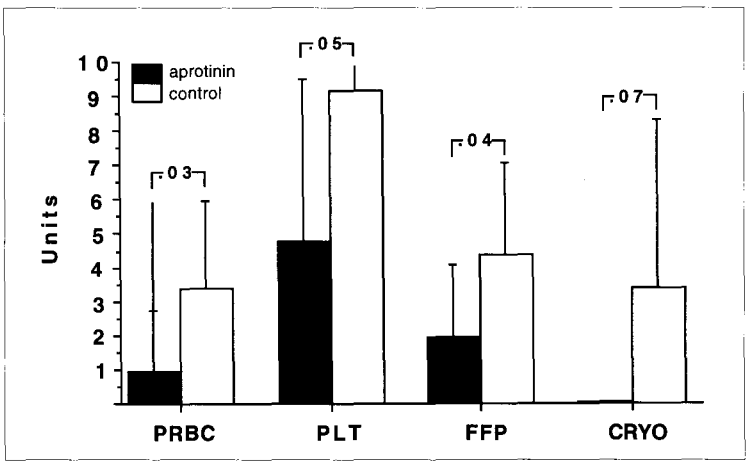
There were no significant differences in total input and output volumes between groups during the

Table 3: Transfusion requirements in ml

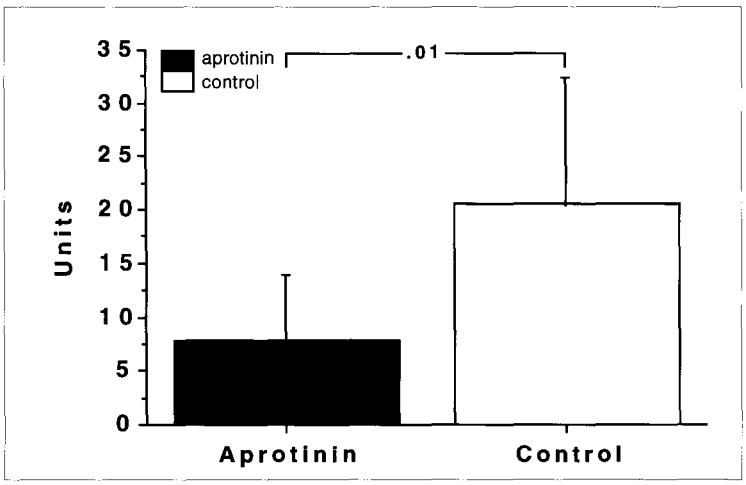
Parameter	Aprotinin Group	Control Group	p Value
Operating Room			
Packed Red Blood Cells	50 ± 141	240 ± 434	NS
Fresh Frozen Plasma	0.0 ± 0.0	450.0 ± 553.5	.03
Platelets	93.8 ± 197.2	336.3 ± 239.8	.05
Cryoprecipitate	0.0 ± 0.0	16.7 ± 38.9	NS
Postoperative Day 1			
Packed Red Blood Cells	173.8 ± 358.3	680.0 ± 501.4	.02
Fresh Frozen Plasma	413.8 ± 492.6	998.3 ± 614.4	.04
Platelets	265.0 ± 250.1	538.0 ± 285.7	.04
Cryoprecipitate	0.0 ± 0.0	33.3 ± 49.2	NS

All data are mean ± SDEV.

Figure 1: 1a. Homologous blood transfusions during hospital stay.



1b. Total homologous blood transfusions during hospital stay.



Legend: CRYO = Cryoprecipitate: FFP = Fresh frozen plasma: PLT = Platelets: PRBC = Packed red blood cells. P values are expressed above histograms. All data is expressed as Mean ± SDEV.

first 24 hours in the ICU. The 12 hour postoperative urine output for both the APR and CTR groups were 1702±830 and 2230±1344 ml (p=ns), respectively. By 24 hours urine output was equal in both groups. Patients in the APR group had a mean ICU ventilator time of 35.3±53.9 hours compared to 13.8±5.8 (p=ns) in the CTR group (Table 5). There was one patient in the former group that had an extended ventilator time due to severe postoperative pulmonary dysfunction who was unsuccessfully weaned from support, and succumbed on the seventh postoperative day. This was the only death in either group. Patients in the APR group were discharged from the hospital an average of 1.2 days before CTR patients (p=.21). Chest tube drainage in the APR group was significantly less than CTR patients during the first 12 postoperative hours and in the total drainage amount (Figure 5).

Table 4: Perioperative laboratory parameters

Parameter	Aprotinin Group	Control Group	p Value
Hemoglobin (G/dl)			
Preoperative	13.1 ± 1.5	13.3 ± 2.1	NS
Postoperative	8.9 ± 0.9	8.7 ± 1.1	NS
Activated Clotting Time (sec)			
Baseline	131.1 ± 10.6	138.2 ± 26.3	NS
Low CPB	475.1 ± 72.7	502.3 ± 91.5	NS
High CPB	663.1 ± 83.8	760.0 ± 170.3	NS
Post CPB	123.1 ± 14.2	141.6 ± 67.5	NS
Prothrombin Time (sec)			
Preoperative	12.6 ± 0.6	12.6 ± 0.7	NS
Postoperative	14.2 ± 1.0	14.4 ± 2.0	NS
Act. Partial Thromboplastin Time (sec)			
Preoperative	29.4 ± 9.9	31.3 ± 8.8	NS
Postoperative	39.9 ± 8.8	27.5 ± 2.4	NS
White Blood Cell Count ($10^3/mm^3$)			
Preoperative	7.8 ± 1.6	8.4 ± 3.7	NS
Postoperative	11.8 ± 3.7	9.4 ± 4.6	NS
Platelet Count ($10^3/mm^3$)			
Preoperative	195.6 ± 58.0	194.4 ± 39.5	NS
Postoperative	116.8 ± 30.5	127.0 ± 28.8	NS
Factor XII (%)			
Preoperative	65.0 ± 9.5	70.0 ± 8.8	NS
Postoperative	38.3 ± 14.6	59.3 ± 33.5	NS
Postoperative Day 1	55.8 ± 11.2	87.1 ± 67.8	NS
Protein C (%)			
Preoperative	90.3 ± 22.0	99.7 ± 20.7	NS
Postoperative	67.1 ± 21.6	59.7 ± 21.1	NS
Postoperative Day 1	66.8 ± 23.6	73.3 ± 14.3	NS
Protein S (%)			
Preoperative	79.0 ± 20.8	76.3 ± 21.1	NS
Postoperative	45.3 ± 14.6	51.0 ± 17.2	NS
Postoperative Day 1	51.2 ± 11.6	54.4 ± 7.4	NS
CH100 (units/ml)			
Preoperative	104.0 ± 82.4	72.6 ± 34.0	NS
Postoperative	68.3 ± 48.7	55.3 ± 20.6	NS
Postoperative Day 1	73.6 ± 77.9	68.6 ± 43.1	NS

Legend: CH100 = Total complement; CPB = Cardiopulmonary bypass. All data are mean ± SDEV.

DISCUSSION

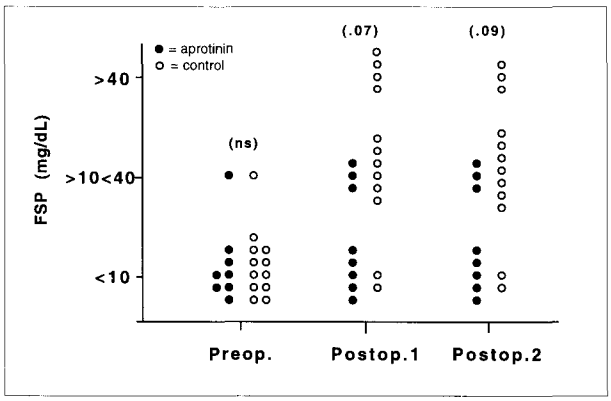
The extracorporealization of blood through synthetic, non-endothelialized circuits generates a whole body inflammatory response, and has been the focus of significant research over the past decade. In the past year two peer reviewed journals have devoted entire issues to this topic[i], which emphasizes both the scientific and clinical interest in this area. The research efforts being performed on CPB induced inflammation is broad ranged. However, two major focal areas of research include altering of the physical properties of the extracorporeal circuit, and examining pharmacological agents that either blunt the systemic activation or protect the tissue and organs most susceptible to injury. The present study was designed to examine the latter by examining the antiinflammatory effects of aprotinin in patients undergoing cardiac surgery with CPB.

The use of aprotinin in cardiac surgery has been well established over the past decade, and its merits in decreasing homologous blood use are unequivocal (22-25). Aprotinin is a single-chain polypeptide with a molecular weight of 6,512 Daltons, consisting of 58 amino acid residues. It is naturally occurring and is isolated from bovine lung tissue. Its main pharmacokinetic actions are based on its ability to inhibit proteases such as trypsin, plasmin and tissue kallikrein (26). Aprotinin's antiprotease activity has also been hypothesized to reduce complement activation which combined with its effect on plasmin and kallikrein, should reduce the systemic inflammatory response (14). Recent experimental evidence suggests that aprotinin may have a beneficial effect of modifying some of the hemodynamic effects associated with endotoxic shock (27). Aprotinin has also been shown to protect against oxidant formation and endothelial cell damage during CPB which preserves myocardial function (28,29). The hemostatic defects resulting from cardiac surgery with CPB result from a number of factors that include both qualitative and quantitative platelet dysfunction, hyperfibrinolytic activity, coagulation deficits and loss of vascular integrity (30-32).

In the present study we chose to use the full Hammersmith loading dose for several reasons. First, it has been shown that a 2 X 10^6 KIU loading dose of aprotinin produces plasma concentrations that effectively inhibit both kallikrein and plasmin (26). Although the hyperfibrinolytic activity associated with CPB is well known (32) this has also been shown to be miti-

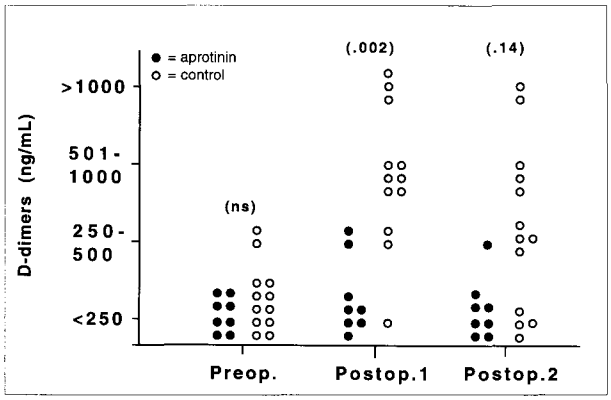
i Cardiopulmonary Bypass: Coagulation and Inflammation Issues. J Cardiovasc Pharm, 1996: 27(Suppl. 1): S1-S92. The Systemic Inflammatory Response to Cardiopulmonary Bypass. Perfusion, 1996; 11: 177-290.

Figure 2. Pre and postoperative fibrin(ogen) split product production.



Legend: APR = Aprotinin group: CTR = Control group: FSP = Fibrin(ogen) split products: Postop. 1 = Postoperative time 1 at chest closure: Postop. 2 = Postoperative time 2 at 24 hours after chest closure: Preop. = Preoperative. P values are expressed above histograms. All data is expressed as Mean ± SDEV.

Figure 3. Pre and postoperative D-dimer production.



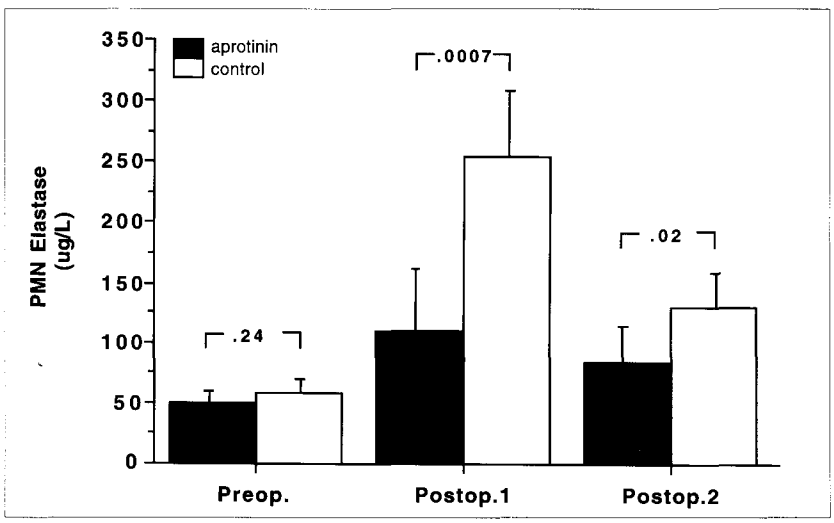
Legend: APR = Aprotinin group: CTR = Control group: Postop. 1 = Postoperative time 1 at chest closure: Postop. 2 = Postoperative time 2 at 24 hours after chest closure: Preop. = Preoperative. P values are expressed above histograms. All data is expressed as Mean ± SDEV.

gated by the use of antifibrinolytic agents such as epsilon-aminocaproic acid and tranexamic acid (10,11). Since we were primarily concerned with aprotinin's influence on inflammation we chose the higher loading dose in an effort to reduce contact activation through kallikrein inhibition. By inhibiting kallikrein, aprotinin may block the conversion of high molecular weight kininogen to the inflammatory mediator bradykinin, as well as inhibit the activation of C1 of the complement system (18). Smaller doses of aprotinin have been shown to result in similar reductions in homologous blood transfusions and chest tube drainage, but not in antiinflammatory activity (5,6,12). We also selected the higher dose because our patient population may be classified as being at a high-risk for bleeding because of the extensive use of preoperative aspirin in our patients.

The antifibrinolytic effects of aprotinin were shown by the lower levels of D-dimers and FSP in the postoperative period. This is in full agreement with others and confirms aprotinin's inhibitory effect on plasmin (31,33). Lu and associates have shown that aprotinin significantly reduced the fibrinolytic markers (FSP, D-dimers) for the first two post-surgical days, but that there was no effect thereafter (33). Mannucci et al have recently demonstrated that alterations in hemostatic parameters following CPB occur up to 30 days after surgery, and that this behavior was not influenced by the administration of aprotinin (31). These authors stated that the continued activation of the coagulation and fibrinolytic systems may be a result of removal of hemostatic plugs formed during and after the surgery.

Protein C and protein S are circulating inhibitors of the coagulation system, with protein C being activated by the endothelial factor thrombomodulin. Thrombomodulin binds to thrombin, which reverses the procoagulant effect of thrombin. The thrombomodulin/thrombin complex activates the protein C system which accelerates its anticoagulant activity (34). Heparin, in large concentrations, has been shown to inhibit the anticoagulant function of the endothelial wall, reducing its thromboresistant characteristics (35). In the present study both pro-

Figure 4. Pre and postoperative polymorphonuclear neutrophil elastase levels.



Legend: Postop. 1 = Postoperative time 1 at chest closure: Postop. 2 = Postoperative time 2 at 24 hours after chest closure: Preop. = Preoperative. P values are expressed above histograms. All data is expressed as Mean ± SDEV.

tein C and protein S decreased during surgery and remained depressed at the first postoperative day, with neither being affected by aprotinin administration, which has been reported by others (36). Although this is in partial agreement with Boldt et al, these authors found a significant difference in protein S levels at the end of surgery which we did not. This can partially be explained by the heparinization protocol which differed between the studies. We chose a loading dose of 300 IU/kg of heparin for both groups while Boldt et al had several heparin regimens that they were comparing, with the aprotinin group in their study receiving 600 IU/kg of heparin. Our study confirmed that of Boldt et al in so far that at the end of the first postoperative day the protein S data were similar in both studies.

Certain organs and tissues are considered to be more susceptible to the injury associated with CPB induced inflammation. One of the most potent mediators in the pathogenesis of post-CPB inflammation and morbidity is the neutrophil (9). Hill and associates have shown that aprotinin reduces the potential for neutrophil-mediated injury by inhibiting adhesion molecule upregulation (6). The adherence of neutrophils to the vascular endothelium creates an environment in which the bioactive neutrophil mediators of injury are placed in close approximation with the susceptible tissue (9). The upregulation of neutrophil surface proteins CD11b and CD18 is shown to be enhanced during CPB (37). One of the most potent proteases released by activated neutrophils is PMN elastase, which degrades structural and functional proteins of human tissue (38). Aprotinin has been shown to protect tissue by reducing neutrophil activation in patients undergoing aortic surgery without CPB (39). We chose to measure PMN elastase, which is released during degranulation of neutrophils, and has been shown to be reduced in patients receiving aprotinin (40). Our data is in agreement with others who have found that aprotinin normalized neutrophil function after major surgery (39,40). The mechanism of action is poorly understood but is more than likely related to the inhibition of both kallikrein and complement activation during CPB (20). In an in vitro study of extracorporeal circulation, Wachtfogel et al have shown that aprotinin, in high doses, inhibited both kallikrein-C1-inhibitor complexes and C1-C1 inhibitor complexes which attenuated the whole body inflammatory response (41). Neutrophil elastase was partially inhibited only at the highest aprotinin dose (0.12 mg/ml). The lower PMN elastase values of the aprotinin patients in the present study were maintained through the first postoperative day. Although it is difficult to determine if there were any direct clinical benefits of reduced neutrophil activation, it seems plausible that the enhanced pulmonary function in the ICU may have been related to these reduced levels.

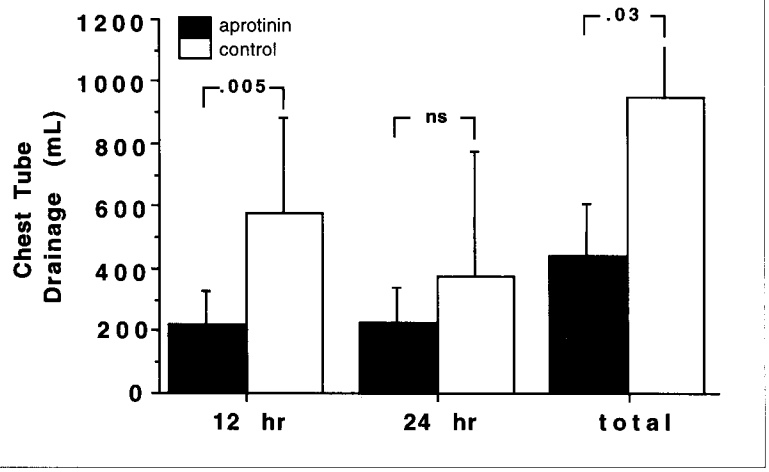
There were no adverse events associated with the use of

Table 5: Intensive care unit parameters

Parameter	Aprotinin Group	Control Group	p Value
Pulmonary Function			
One Hour in ICU			
$PaCO_2$ (mmHg)	37.1 ± 4.8	43.3 ± 7.1	.05
PaO_2 (mmHg)	176.4 ± 54.2	156.9 ± 74.5	NS
MAP (cm H_20)	8.3 ± 1.5	10.8 ± 2.9	.03
FIO_2 (%)	0.63 ± 0.18	0.75 ± 0.20	NS
12 Hours Postop			
$PaCO_2$ (mmHg)	38.4 ± 2.9	35.7 ± 6.3	NS
PaO_2 (mmHg)	104.4 ± 17.3	95.9 ± 30.5	NS
MAP (cm H_20)	7.9 ± 1.7	10.9 ± 3.1	.03
FIO_2 (%)	0.50 ± 0.22	0.48 ± 0.11	NS
ICU Ventilator Time (hours)	35.3 ± 53.9	13.8 ± 5.8	NS
median	17.9	12.8	
ICU Stay (hours)	43.3 ± 51.0	41.3 ± 19.1	NS
median	24.5	19.1	
Total Hospital Stay (days)	6.1 ± 0.8	7.3 ± 2.5	NS
Mortality	1/8	0/12	NS

Legend: ICU = Intensive care unit: MAP = Mean airway pressure
All data are mean ± SDEV.

Figure 5. Postoperative chest tube drainage.



P values are expressed above histograms. All data is expressed as Mean ± SDEV.

aprotinin. Although we did not measure renal function through laboratory analysis in the postoperative period, we found no significant differences in urine output in either the control or treatment group. No patient in either group suffered neurological injury or renal compromise during the study.

It is important to realize that the extracorporeal process is not the sole modulator of the inflammatory response seen in cardiac surgical patients. Ischemia, hypoperfusion, reperfusion phenomena, the mechanical type of perfusion (pulsatile vs. nonpulsatile), temperature changes, hemoglobin levels, and blood transfusion all play significant roles (27). The significant reduction in homologous blood transfusions in patients receiving aprotinin in the present study probably attenuated some of the inflammation as a secondary benefit of the drug.

In conclusion, the use of full dose aprotinin resulted in significant improvements in patient outcomes as seen in fewer homologous transfusions, reduced chest tube drainage, lowered polymorphonuclear neutrophil elastase levels, and improved pulmonary function. All of which support the benefits of aprotinin in attenuating the inflammatory responses associated with cardiac surgery and cardiopulmonary bypass.

ACKNOWLEDGEMENTS

The authors wish to thank Bayer Pharmaceuticals, West Haven, CT, for providing us with funding for this study. We also thank Li Chen Hsu, Ph.D., of Baxter Healthcare, Irvine, CA, for providing us with the elastase kits. The participation in the study of the following individuals is greatly appreciated: Nancy Abrahms, MSAT, Marco Vasquez, BS, CCP, Andrew Bonness, BS, Chad Brady, BS, Suzanne Ficek, MS, Susan Miyeno, Linda Fell, MS, MSAT.

REFERENCES

1. Chenoweth DE, Cooper SW, Hugli TE, Stewart RW, Blackstone EH, Kirklin JW. Complement activation during cardiopulmonary bypass. N Eng J Med. 1981; 304:497-503.
2. Svennevig JL, Geiran OR, Karsen H, et al. Complement activation during extracorporeal circulation. In vitro comparison of Duraflo II heparin-coated and uncoated oxygenator. J Thorac Cardiovasc Surg. 1993; 106: 466-72.
3. Edmunds LH. Blood-surface interactions during cardiopulmonary bypass. J Card Surg. 1993;8:404-10.
4. Allen SM. Leukocyte depletion in cardiothoracic surgery. Perfusion. 1996; 11: 270-7.
5. Hill, GE, Pohorecki R, Alonso A, Rennard SI, Robbins, RA. Aprotinin reduces interleukin-8 production and lung neutrophil accumulation after cardiopulmonary bypass. Anesth Analg. 1996;83:696-700.
6. Hill GE, Alonso A, Spurzem RJR, Stammers AH, Robbins RA. Aprotinin and methylprednisolone equally blunt cardiopulmonary bypass-induced inflammation in humans. J Thorac Cardiovasc Surg. 1995;110:1658-62.
7. Takaori M, Fukui A, Kimura K, et al. Hemostatic mechanism associated with aprotinin during and after extracorporeal circulation for cardiac and great vessel surgery. Masui. 1995; 44:1661-6.
8. Wendel HP, Heller W, Gallimore MJ, Hoffmeister HE. Heparin-coated oxygenators significantly reduce contact system activation in an in vitro cardiopulmonary bypass model. Blood Coagul Fibrinolysis. 1994;5:673-8.
9. Cameron D. Initiation of white cell activation during cardiopulmonary bypass: Cytokines and receptors. J Cardiovasc Pharmacol. 1996;27(Suppl. 1):S1-5.
10. Daily PO, Lamphere JA, Dembitsky WP, et al. Effect of prophylactic epsilon-aminocaproic acid on blood loss and transfusion in patients undergoing first-time coronary artery bypass grafting. A randomized double-blind study. J Thorac Cardiovasc Surg. 1994; 108:99-108.
11. Horrow JC, Van Riper DF, Strong MD, et al. Hemostatic effects of tranexamic acid during cardiac surgery. Circulation. 1991;84: 2063-70.
12. Speekenbrink RG, Wildevuur CR, Sturk A, Eijsman L. Low-dose and high-dose aprotinin improve hemostasis in coronary operations. J Thorac Cardiovasc Surg. 1996;112: 523-30.
13. Lemmer JH, Dilling EW, Morton JR, et al. Aprotinin for primary coronary artery bypass grafting: a multicenter trial of three dose regimens. Ann Thorac Surg. 1996;62: 1659-67.
14. Royston D. Serine protease inhibition prevents both cellular and humoral responses to cardiopulmonary bypass. J Cardiovasc Pharmacol. 1996;27(Suppl. 1):S42-9.
15. Mohr R, Goor DA, Lusky A, Lavee J. Aprotinin prevents cardiopulmonary bypass-induced platelet dysfunction. A scanning electron microscope study. Circulation. 1992;86: II405-9.
16. Marx G, Pokar H, Reuter H, Doering V, Tilsner V. The effects of aprotinin on hemostatic function during cardiac surgery. J Cardiothorac Vasc Anesth. 1991;5:467-74
17. Fuhrer G, Gallimore MJ, Heller W, Hoffmeister HE. Aprotinin in cardiopulmonary bypass-effects on the Hageman factor (FXII)—Kallikrein system and blood loss. Blood Coagul Fibrinolysis. 1992;3:99-104.
18. Davis R, Whittington R. Aprotinin. A review of its pharmacology and therapeutic efficacy in reducing blood loss associated with cardiac surgery. Drug Evaluation. 1995; 49:954-83.
19. Becket MA, Brannan J, Fleming J, Taylor KM. The effect of high dose aprotinin therapy on the systemic inflammatory response in a porcine model of cardiopulmonary bypass. Perfusion. 1996;11: 278-80.
20. Wachtfogel YT, Hack CE, Nuijens JH, et al. Selective kallikrein inhibitors alter human neutrophil elastase release during extracorporeal circulation. Am J Physiol. 1995; 268: H1352-7.

21. Seghaye MC, Duchateau J, Grabitz RG, et al. Influence of low-dose aprotinin on the inflammatory reaction due to cardiopulmonary bypass in children. Ann Thorac Surg. 1996; 61:1205-11.
22. Royston D, Bidstrup BP, Taylor KM, Sapsford RN. Effect of aprotinin on need for blood transfusion after repeat open-heart surgery. Lancet. 1987;2: 1289-91.
23. Levy JH, Pifarre R, Schaff HV, et al. A multicenter, double-blind, placebo-controlled trial of aprotinin for reducing blood loss and the requirement for donor-blood transfusion in patients undergoing repeat coronary artery bypass grafting. Circulation. 1995;92: 2236-44.
24. Schonberger JP, Woolley S, Tavilla G, et al. Efficacy and safety of a blood conservation program including low-dose aprotinin in routine myocardial revascularization. J Cardiovasc Surg Torino. 1996;37: 35-44.
25. Shinfeld A, Zippel D, Lavee J, et al. Aprotinin improves hemostasis after cardiopulmonary bypass better than single-donor platelet concentrate. Ann Thorac Surg. 1995;59 :872-6.
26. Levy JH, Bailey JM, Salmenpera M B. Pharmacokinetics of aprotinin in preoperative cardiac surgical patients. Anesthesiology 1994; 80:1013-18.
27. Taylor KM. Improved outcome for seriously ill open heart patients: Focus on reoperation and endocarditis. J Heart Lung Transplant. 1993;1 2:S14-8.
28. Rao PS, Palazzo RS, Bocchieri KA, et al. Aprotinin protects against myocardial and oxidant formation and endothelial cell damage during open heart surgery. Ann N Y Acad Sci. 1996;793:514-6.
29. Broche VF, Suarez AR, Olembre E, et al. Aprotinin effects related to oxidative stress in cardiosurgery with mechanical cardiorespiratory support (CMCS). Ann NY Acad Sci. 1996;793:521-4.
30. Woodman RC, Harker LA. Bleeding complications associated with cardiopulmonary bypass. Blood. 1990; 76:1680-97.
31. Mannucci L, Gerometta PS, Mussoni L, et al. One month follow-up of haemostatic variables in patients undergoing aortocoronary bypass surgery. Effect of aprotinin. Thromb Haemost. 1995;73:356-61.
32. Stammers AH, Rasmussen CA, Kratz JM. Hemorrhagic effects of post cardiopulmonary bypass fibrinolysis. J Extra-Corpor Technol. 1993;25:133-9.
33. Lu H, Du-Buit C, Soria J, et al. Postoperative hemostasis and fibrinolysis in patients undergoing cardiopulmonary bypass with or without aprotinin therapy. Thromb Haemost. 1994;72: 438-43.
34. Boldt J, Schindler E, Welters I, et al. The effect of the anticoagulation regimen on endothelial-related coagulation in cardiac surgery patients. Anaesthesia. 1995;50:954-60.
35. Fresbelius S, Nydahl S, Swedenborg J. Coagulant and non-coagulant thrombin enzymatic activity on the endothelium. Blood Coag Fibrin. 1990;1:285-92.
36. Boldt J, Schindler E, Knothe C, et al. Does aprotinin influence endothelial-associated coagulation in cardiac surgery? J Cardiothorac Vasc Anesth. 1994;8:527-31.
37. Gillinov AM, Redmond JM, Zehr KJ, et al. Inhibition of neutrophil adhesion during cardiopulmonary bypass. Ann Thorac Surg. 1994;57:126-33.
38. Murata A, Toda H, Uda K, et al. Protective effect of recombinant neutrophil elastase inhibitor (R-020) on sepsis-induced organ injury in rat. Inflammation. 1994;18:337-47.
39. Lord RA, Roath OS, Thompson JF, Chant AD, Francis JL. Effect of aprotinin on neutrophil function after major vascular surgery. Br J Surg. 1992;79:517-21.
40. van Oeveren W, Jansen NJG, Bidstrup BP, et al. Effects of aprotinin on hemostatic mechanisms during cardiopulmonary bypass. Ann Thorac Surg. 1987;44:640-5.
41. Wachtfogel YT, Kucich U, Hack CE, et al. Aprotinin inhibits the contact, neutrophil, and platelet activation systems during simulated extracorporeal perfusion. J Thorac Cardiovasc Surg. 1993;106:1-9.