
Original Article***Co-administration of Aprotinin and Epsilon–Aminocaproic Acid During Cardiopulmonary Bypass in a Swine Model***

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Keywords: cardiopulmonary bypass, aprotinin, epsilon–aminocaproic acid, fibrinolysis, thrombosis

Presented at the 37th International Meeting of the American Society of Extra-Corporeal Technology, April, 1999, New Orleans, Louisiana

Recipient: American Society of Extra-Corporeal Research Grant, 1998–1999.

ABSTRACT

Despite the beneficial effects of pharmacological interventions to prevent bleeding and to reduce the need for autogeneic blood, there are concerns that these agents induce a prothrombotic state. The purpose of this study was to examine the coagulation phenomena influenced by the coadministration of epsilon–aminocaproic acid (EACA) and aprotinin during cardiopulmonary bypass (CPB).

A swine model of CPB was utilized in this study. During 120 min of CPB, treatment animals ($N = 5$) received 6×10^6 Kiu of aprotinin and 30 grams of EACA; whereas, control animals ($N = 3$) received an equal volume of 0.9 % saline. Indices of thrombogenicity included hematological variables, gross pathology, and circuit examination for the presence of thrombus.

The application of both antifibrinolytics resulted in an increase use of heparin. Total heparin requirements were significantly different between treatment group ($58,800 \pm 3493$ iu) versus control group ($51,000 \pm 3464$ iu). D-dimer concentration was also significantly higher in the control group ($500\text{--}1000$ ng mL⁻¹) than in the treatment group ($250\text{--}500$ ng mL⁻¹) at 5 and 30 min postprotamine. Other coagulation markers tested were not observed to be statistically significant between groups. Thromboelastographic (TEG) index decreased in the treatment group during the surgical procedure and bypass from 2.74 ± 2.9 to -1.36 ± 4.1 as compared to an increase from 2.62 ± 2.9 to 4.05 ± 0.4 in the control group. Pathologic analysis revealed occurrences of thrombus formation in small vessels in the lung and kidney glomeruli of treatment animals. The concurrent use of both aprotinin and EACA may induce a prothrombotic or coagulant state as determined by histological assessment.

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INTRODUCTION

Postoperative hemorrhage following cardiac surgery with cardiopulmonary bypass (CPB) continues to be one of the most important contributors to patient morbidity and mortality despite increased efforts to reduce hemostatic alterations and blood loss. Activation of hemostasis is well recognized in patients undergoing cardiac operations with CPB (1). Cardiopulmonary bypass involves extensive contact between blood and artificial surfaces of the extracorporeal circuit (ECC) that produces activation of coagulation. Hemostatic derangements occurring when blood is exposed to the ECC may lead to a heightened thrombin generation leading to thrombosis.

Thrombin is a potent stimulant for the release of tissue plasminogen activator (tPA) from vascular endothelium, and fibrin potentiates the generation of plasmin from the zymogen plasminogen (2). The stimulation of the fibrinolytic system leads to inadequate clot formation and bleeding, which often requires homologous blood transfusion (3). Numerous investigations have identified increased fibrinolytic activity in association with cardiac surgery. Tanaka and colleagues demonstrated a direct relationship between plasma concentrations of tPA and duration of CPB (4). Other investigators have observed increased concentrations of fibrin(ogen) degradation products (FDP) and D-dimer (DD) during CPB (5). Further investigations have demonstrated that substantial concentrations of thrombin and fibrin are generated throughout the period of CPB (6, 7).

Current methods used by clinicians to reduce postoperative bleeding and blood transfusion include heparin-coated circuitry, autologous plasmapheresis, autotransfusion with or without cell-saving techniques, and the administration of antifibrinolytic agents (8). Pharmacological intervention has been instrumental in the prevention of hemostatic defect and reducing the extent of excessive bleeding associated with CPB. Currently, three antifibrinolytic drugs are available for clinical administration: the synthetic lysine analogues, epsilon-aminocaproic acid (EACA) and tranexamic acid (TA), and the natural serine protease inhibitor, aprotinin. The safety and efficacy of aprotinin, EACA, and TA have been closely examined (9–11); however, there are no data to suggest that they should be combined with CPB. There is a concern that the combination of these agents will induce a prothrombotic or coagulant state.

Experiments using combinations of aprotinin and TA has been reported by Longstaff (12). His results support the idea that a combination therapy of aprotinin and TA may be effective and, therefore, provides a slight synergistic effect. Because EACA and TA are both lysine analogues, a synergistic effect may also be observed with the concurrent application of EACA and aprotinin. However, the fact that each medication has discrete mechanisms of activity may also reverse the hemorrhagic state and produce an increased thrombogenic capacity because of the stimulation of thrombin.

Adding a lysine analogue has been shown to minimize the increase in primary fibrinolysis that occurs during CPB (13–15). The antifibrinolytic effect of EACA is related mainly to its ability to form a reversible complex with plasminogen. The location of inhibition are the lysine binding sites of plasmin(ogen), which is the domain responsible for binding to fibrin. Lysine binding sites are also where antifibrinolytic aminoacids (α_2 -antiplasmin) attach. As with the synthetic antifibrinolytics, aprotinin inhibits plasmin and plasminogen. The primary benefits of aprotinin have been attributed to its ability to minimize peri- and postoperative blood loss during cardiac surgery. This is a result of the complex pharmacologic nature of this medication in affecting numerous processes involved in both hemostasis and inflammation. Aprotinin has various effects that pertain specifically to the components of platelet preservation, the intrinsic pathway, the inflammatory cascades, and fibrinolysis (16–18).

The purpose of this study was to examine the coagulation phenomena associated with the co-administration of aprotinin and EACA during CPB. To the author's knowledge, no data exist to refute or suggest that the combination of aprotinin and EACA may be beneficial. Because each type of medication has a specific mechanism for activity, it can be speculated that the co-administration of these agents may have profound effects on the homeostatic potential and may result in a hypercoagulable state.

MATERIALS AND METHODS

ANIMAL PROTOCOL

The research protocol was approved by the Institutional Animal Care Utilization Committee at the University of Nebraska Medical Center (UNMC). All animals used in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

All procedures were conducted as acute studies on 70-kg swine anesthetized with a mixture of ketamine (20 mg kg⁻¹) and xylazine (2 mg kg⁻¹) given intramuscularly. Each animal was intubated with a 6.5 F endotracheal tube and ventilated with a tidal volume (20–30 mL kg⁻¹) at a rate of 15–20 breaths per min. Electrocardiogram leads were placed, and the heart rate was continuously monitored. The femoral artery and vein were cannulated with 14-gauge needles for hemodynamic monitoring as well as medication infusion. Animals were intermittently dosed with pentothal (100 μ g kg⁻¹) and pavulon (50 μ g kg⁻¹). Bretylium (3 mg kg⁻¹) was administered before midline sternotomy.

A midline sternotomy was performed, and the great vessels were dissected free in preparation for cannulation. A purse string suture was placed in the aorta for arterial cannulation. Before cannulation, the animal received a bolus dose of 400 iu kg⁻¹ of bovine lung heparin and adequate anticoagulation

(>480 sec) assured via kaolin-based assays. A 7.0-mm soft flow arterial cannula^a was used. A second purse string suture was placed in the right atrial appendage to prepare for venous cannulation. The venous cannula was a dual stage^b 29/37 or 34/46 F. The circulating heparin concentration was maintained greater than 2.5 mg mL⁻¹ as determined by heparin-protamine titration.^c

CARDIOPULMONARY BYPASS

The experimental period consisted of a CPB duration of 120 minutes. A standard ECC was utilized that consisted of a hollow fiber membrane oxygenator,^d an integrated hard shell cardiomy/venous reservoir,^e a 40-micron arterial line filter,^f polyvinylchloride tubing, and a twin head positive displacement roller pump.^g The circuit was primed with 1200 mL of Plasmalyte-A, 50 mL of 8.4 % sodium bicarbonate (1 mEq mL⁻¹), and 2500 iu L⁻¹ of bovine lung heparin. The perfusate temperature was reduced to 32°C following the initiation of CPB and maintained at that level throughout the experiment, up until rewarming. After terminating CPB, heparin was reversed with protamine sulfate at a rate of 1 mg of protamine per 100 iu of total heparin administered. Adequacy of reversal was ascertained via heparin-protamine titration measurement and activated clotting time^h (ACT).

Each animal was maintained within normal physiologic and hemodynamic parameters. A mean arterial blood pressure (50–80 mm Hg) was maintained with the use of vasoactive substances (neosynephrine, 80 µg mL⁻¹ or sodium nitroprusside 200 µg mL⁻¹). In addition, the following parameters were controlled: central venous pressure (0–8 mmHg), arterial acid-base management according to alpha-stat techniques (pH: 7.35–7.45, PaCO₂: 35–45 mmHg, PaO₂: 100–150 mmHg, HCO₃: 22–26 mMol), anticoagulation (Kaolin-ACT > 480 sec) and hematocrit (20–25 %). An in-line blood gas monitorⁱ was used to maintain venous saturation between 65–75 %. Arterial blood gases, mixed venous saturation, electrocardiogram, and hemodynamics were measured continuously.

At the termination of each experiment, the animal was euthanized after the last blood samples were obtained after 30 min postprotamine administration. Barbiturate (1 mg kg⁻¹) and potassium chloride (20 mEq) were directly administered into the aortic root. The ventilator was turned off, and the endotracheal tube was removed. If electrical activity ensued or spontaneous breathing occurred, the animal was given an additional dose of barbiturate and potassium chloride.

EXPERIMENTAL PROTOCOL

Animals were randomized the day before the experiment to either the treatment ($n = 5$) or control ($n = 3$) group. Animals in the treatment group received 1 mL test dose of aprotinin^j (10,000 Kiu) 10 min before chest incision to identify hypersensitivity (reduction in mean blood arterial pressure by 50 %). The dosage regimen for the treatment group was as follows: a loading dose of 2–10⁶ Kiu of aprotinin over a 15-min period was given before chest incision along with 10 g of EACA^k; 2–10⁶ Kiu was added to the pump prime along with 10 g of EACA; and a continuous infusion of 5–10⁵ Kiu h⁻¹ was given throughout the experiment, terminating 20 min postprotamine infusion, with an additional 10 g of EACA administered post-protamine infusion. Pigs in the control group received equal amounts of 0.9 % saline.

BLOOD SAMPLE COLLECTION

Blood samples were drawn for coagulation assay studies of the effects of median sternotomy and CPB on systemic thrombogenic and fibrinolytic activity. Samples for determination of the hemostatic variables were taken from the femoral arterial catheter after 10 mL of blood was withdrawn to assure proper clearance of heparin. For the plasma tests, blood drawn was anticoagulated with 4.5 mL whole blood to 0.5 mL of 130 mM sodium citrate. Blood samples (20 mL) were taken at four time points: presternotomy (baseline), poststernotomy, 5 min post-protamine, and 30 min postprotamine. In addition to the four time points, additional blood samples were collected at 40 and 80 min CPB for whole blood analysis with the ACT, high thrombin time^l (HiTT), thromboelastograph^m (TEG), and heparin-protamine titration. TEG parameters were recorded by the computer and then incorporated into an equation to calculate TEG indices. A higher TEG index is indicative of increased clotting activity.

CIRCUIT EVALUATION

After 120 min of CPB and the administration of protamine, the animal was euthanized according to the animal protocol. The circuit was examined for the presence of gross thrombus by draining blood from the ECC through a Buchner funnel with a 25 micron, 32 cm coarse filter at a vacuum pressure of –100 mmHg. Following removal of the perfusate, the circuit was rinsed and examined for residual thrombi at the following locations: membrane oxygenator, integrated hard shell venous reservoir, and arterial line filter. Gross thrombus was defined as any solid substance greater than 50 mm in length adhering to the ECC. The total number and geometry of thrombi were quantified and compared between groups.

^a Terumo Sarns, Ann Arbor, MI

^b Edwards LifeSciences., Midvale, UT

^c Hepcon/HMS, Medtronic, Minneapolis, MN

^d Vision, Gish Biomedical, Irvine, CA

^e Gish Biomedical, Irvine, CA

^f Medtronic, Eden Prairie, MN

^g Terumo Sarns, Ann Arbor, MI

^h Hemochron 800, International Technidyne, Edison, NJ

ⁱ CDI 300, Terumo Sarns, Ann Arbor, MI

^j Trasylol, Bayer Corporation, West Haven, CT

^k Aminocaproic Acid, American Regent Laboratories, Shirley, NY

^l Hemochron 8000, International Technidyne, Edison, NJ

^m Haemoscope Corporation, Skokie, IL

HISTOLOGICAL ANALYSIS

The presence of thrombus in the animal was ascertained by sectioning heart, lung, and kidney immediately post euthanasia. The trachea was cannulated, and 10 % formalin was infused into the respiratory tract before dissection was undertaken. Distention with 10 % formalin was carried out via the bronchi. The resected end was clamped with hemostats, and the specimen was immersed in fixative for 12–24 h to obtain good histology. After fixation, the specimens were sliced, and blocks were taken for histologic processing. Two 2–3-cm sections were obtained from both heart and kidney each, and four 2–3-cm sections were obtained from the lungs. Pathological analysis for the presence of fibrin, platelet–leukocyte aggregates, and intravascular clot formation was made by the UNMC Clinical Pathology Laboratory.

LABORATORY ANALYSIS

Coagulation markers provide a sensitive and specific means of quantifying fibrinolytic and thrombotic activity. The tests included plasminogen activator inhibitor-1 (PAI-1), tPA, prothrombin fragment 1.2 (PF 1.2), thrombin–antithrombin III complex (TAT), and DD concentration. Blood samples were kept at 4°C until all specimens were collected and then transported to the laboratory. All specimens were centrifuged at 3000 g for 10 min. At the end of the centrifugation process, the plasma was removed, allocated, and stored frozen at –70°C until assayed.

All determinations (standards, control, and samples) were performed in duplicate. The reference curve was established for each series of measurements using the standards. Thrombin generation was assessed by the measurement of TAT and PF 1.2, using an enzyme-linked immunosorbent assay (ELISA).ⁿ The tPA ELISA^o kit was utilized to detect disorders of the fibrinolytic system, and PAI-1 ELISA^o kit was used for the quantitative determination of PAI-1 antigen in plasma. A latex agglutination assay^p for the semiquantitative determination of fibrin DD in plasma or serum was utilized.

STATISTICAL ANALYSIS

All data were recorded and entered into a computer database. In addition, data for ELISAs were corrected for hemodilution. Statistical analysis was performed utilizing a commercially available statistics program.^q Parametric data were analyzed using one way analysis of variance (ANOVA). When significant F ratios were reached, additional multiple comparison tests were performed, which included Fisher's Least Significance Difference (intergroup comparison) and Dunnett's Two-tailed Test of Significance (intragroup comparison). Fisher's Least Significance Difference was performed for small

samples with ≤ 5 observations. Statistical significance was accepted at the $p < .05$ level. All data are presented as mean \pm standard deviation of the mean.

RESULTS

DEMOGRAPHY AND PERIOPERATIVE DATA

Eight swine were used in the investigation. The operative data between the two groups are described in Table 1. Both groups were similar with respect to weight, length, and body surface area. There was a significant difference ($p = .022$) between the treatment and control groups with regard to the total heparin administered throughout bypass ($58,000 \pm 3493$ iu vs. $51,000 \pm 3464$ iu). However, the total protamine dose used to reverse heparin was not significant.

ACTIVATING CLOTTING TIME AND HIGH THROMBIN TIME

ACT for both groups were maintained above 480 sec during bypass. Although not significant, ACT was higher in the treatment group as compared to control at 40 and 80 min CPB. Circulating heparin concentration was 1.90 ± 0.2 mg kg⁻¹ in the treatment animals and 2.17 ± 0.3 mg kg⁻¹ in the control at 40 min of bypass ($p = \text{NS}$). In addition, the circulating heparin at 80 min CPB was 2.10 ± 0.4 mg kg⁻¹ in the treatment animals and 2.50 ± 0.0 mg kg⁻¹ in the control group ($p = \text{NS}$).

HiTTs are depicted in Figure 1. In the treatment group, the HiTT was 346.0 ± 162.6 seconds versus to 1000 ± 0.0 sec in the control at 40 min CPB ($p = .03$). There is no error bar on the bar graph in the control group at 40 min CPB, because HiTT greater than 1000 sec were recorded as 1000 sec for statistical analysis. Significant difference was not observed with HiTT at 80 min CPB with 754.2 ± 267.1 sec in the treatment animals versus to 871.0 ± 223.4 sec in control group.

THROMBOELASTOGRAPHY

There was no significant difference between the treatment and control groups in regard to individual TEG parameters. Figure 2 illustrates the TEG Index. The TEG Index increased in the control group from baseline (2.62 ± 2.9) to 80 min CPB (4.05 ± 0.4), and declined 30 min postprotamine (3.47 ± 1.0). In the treatment group, the TEG Index decreased from baseline

Table 1: Operative data.

	Aprotinin and EACA (n = 5)	Control (n = 30)	p-value
Weight (kg)	72.8 \pm 5.9	68.3 \pm 2.9	NS
Length (cm)	121.5 \pm 5.0	118.8 \pm 3.3	NS
Body surface area (m ²)	1.6 \pm 0.1	1.5 \pm 0.0	NS
Total heparin (iu)	58,800 \pm 3,493	51,000 \pm 3,464	0.022
Total protamine (mg)	207.0 \pm 40.5	196.0 \pm 6.9	NS

ⁿ American Diagnostica Inc., Greenwich, CT

^o Bering Diagnostica Inc., Westwood, MA

^p Sigma Diagnostics ACCUCLOT™ D-dimer, St. Louis, MO

^q Super ANOVA, Abacus Concepts, Berkeley, CA

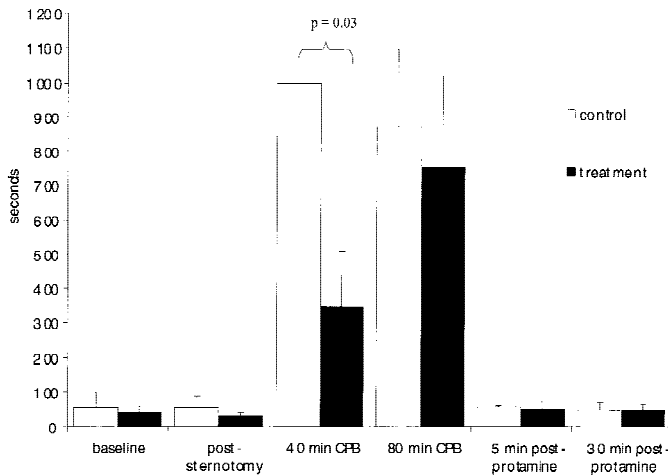


Figure 1: High thrombin time.

(2.74 ± 3.0), during bypass, and 30 min postprotamine (-1.36 ± 4.1). There was a significant difference observed between treatment and control groups at 80 minutes CPB ($p = .02$).

TISSUE PLASMINOGEN ACTIVATOR

Although there were slight elevations in tPA concentration (Figure 3a) in the control versus treatment group, no significant differences were observed between groups. In the control group, tPA decreased from baseline ($2.12 \pm 1.4 \text{ ng mL}^{-1}$), during bypass, and 30 min postprotamine ($1.77 \pm 1.3 \text{ ng mL}^{-1}$). In contrast, tPA increased slightly from baseline ($1.54 \pm 0.8 \text{ ng mL}^{-1}$) to poststernotomy ($1.56 \pm 0.8 \text{ ng mL}^{-1}$) in the treatment group, and then decreased during bypass and after protamine administration.

PLASMINOGEN ACTIVATOR INHIBITOR-1

PAI-1 in Figure 3b increased 76 % above baseline in the treatment group and peaked at 5 min postprotamine. In addition, PAI-1 decreased after 5 min protamine administration; however, it remained 59 % above baseline at 30 min postprotamine. There was a significant difference between baseline ver-

sus 5 min postprotamine in the treatment group ($p = .009$). In the control group, PAI-1 increased 21 % above baseline at poststernotomy, and remained 8 % above baseline at 5 and 30 min postprotamine.

PROTHROMBIN FRAGMENT 1.2

Figure 3c depicts concentrations of PF 1.2. In the treatment group, PF1.2 increased at poststernotomy ($0.06 \pm 0.03 \text{ nmol L}^{-1}$), decreased during bypass, and peaked 30 min after protamine administration ($0.08 \pm 0.07 \text{ nmol L}^{-1}$). Levels in the control group peaked at poststernotomy ($0.047 \pm 0.03 \text{ nmol L}^{-1}$), and remained slightly elevated in the postprotamine period. At all measured times, there were no significant differences in the production of PF 1.2 between the treatment and placebo groups, indicating that aprotinin and/or EACA had not changed the degree of thrombin generated during and after CPB.

THROMBIN/ANTITHROMBIN III COMPLEX

TAT levels (Figure 3d) in the treatment group were slightly higher versus control group at baseline, poststernotomy, and 5 min postprotamine; however, the differences were not significant. Levels in the treatment group increased during CPB, peaked at 5 min postprotamine ($53.75 \pm 34.8 \text{ } \mu\text{g L}^{-1}$), and decreased to $46.42 \pm 19.5 \text{ } \mu\text{g L}^{-1}$ at 30 min postprotamine. TAT levels in the control group also increased after sternotomy ($19.85 \pm 2.3 \text{ } \mu\text{g L}^{-1}$), 5 min postprotamine, and peaked at 30 min postprotamine ($47.13 \pm 10.5 \text{ } \mu\text{g L}^{-1}$). No significant differences were observed at each time point between treatment and control groups.

D-DIMER

Figure 4 illustrates DD concentrations between treatment and control group. There was no significant difference between DD levels within the treatment and control groups at baseline and poststernotomy. However, significant difference was observed between the two groups at 5 and 30 min postprotamine ($p = .0001$).

CIRCUIT ANALYSIS

Following euthanasia of the swine and drainage of the perfusate from the ECC, examination of the ECC revealed no thrombus formation in the membrane oxygenator, integrated cardiotomy/venous reservoir, or arterial filter.

HISTOLOGICAL ANALYSIS

The definition of ‘rare’ used in this section is defined as a total occurrence of 1–3 thrombi. If there were >3 occurrences, it was defined as ‘many.’ Histological analysis revealed occurrences of many thrombi in vessels $<100 \text{ } \mu\text{m}$ in the lung of treatment animals. Rare occurrences of thrombi were also detected in lung of control animals. Figures 5 and 6 illustrate examples of thrombi in both groups. Many clots were also found in glomeruli of kidney specimens of nearly all treatment

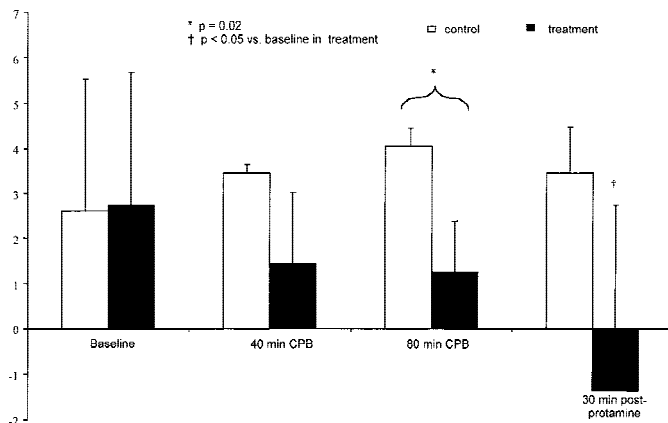


Figure 2: Thromboelastograph Index.

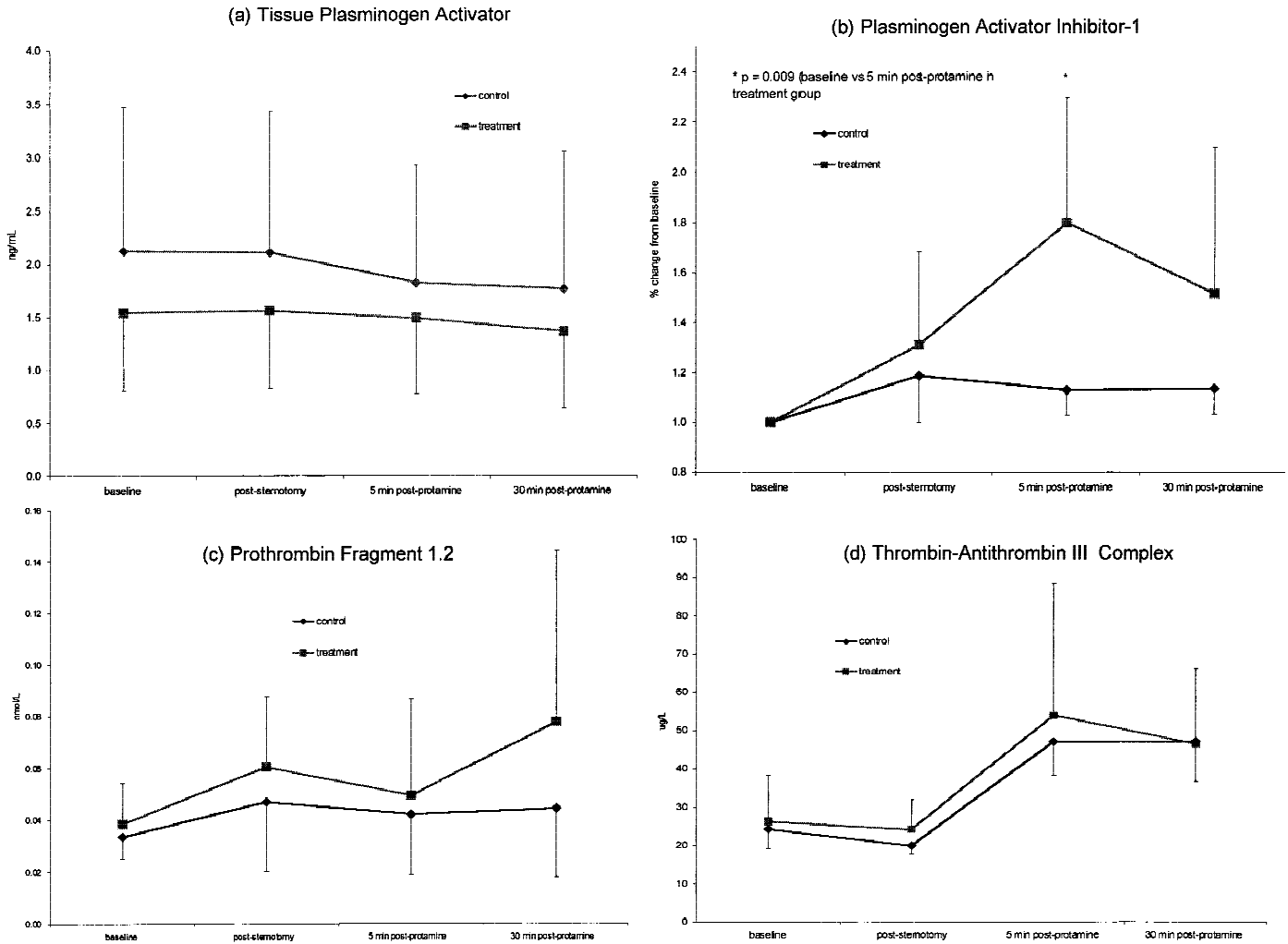


Figure 3:

animals and rare in the control animals (Figures 7 and 8). A rare thrombus was found in a heart tissue sample in one control animal (Figure 9), and a rare heart thrombus was discovered from one treatment animal (Figure 10). There was no significant difference between treatment and control group for thrombus formation in the heart, kidney, or lung. However, the aggregate thrombus appearance was significantly higher in treatment animals ($p < .05$).

DISCUSSION

Both cardiac surgery and the extracorporeal circulation of blood result in a stimulation of plasminogen activators that enhance hyperfibrinolysis. When unchecked, this results in excessive plasmin activation, which destabilizes the fibrin clot and leads to excessive hemorrhage. The normal function of the fibrinolytic system is to modulate the generation of fibrin so that thrombosis with subsequent vessel occlusion does not occur. tPA serves as an intrinsic and extrinsic source for plasmin activation and is released by the endothelium during cardiac

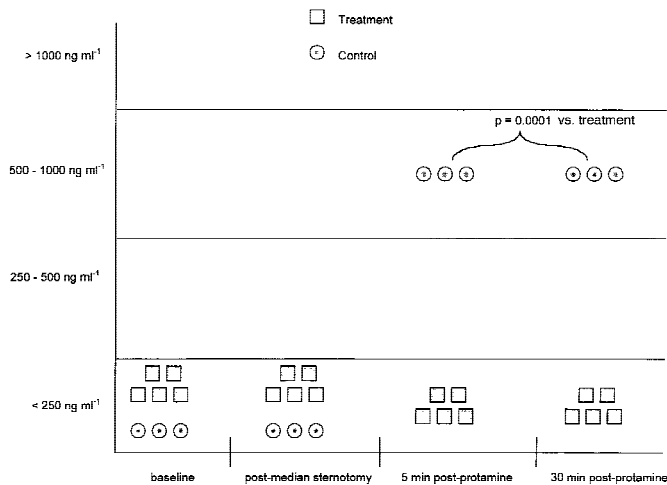


Figure 4: D-Dimer.

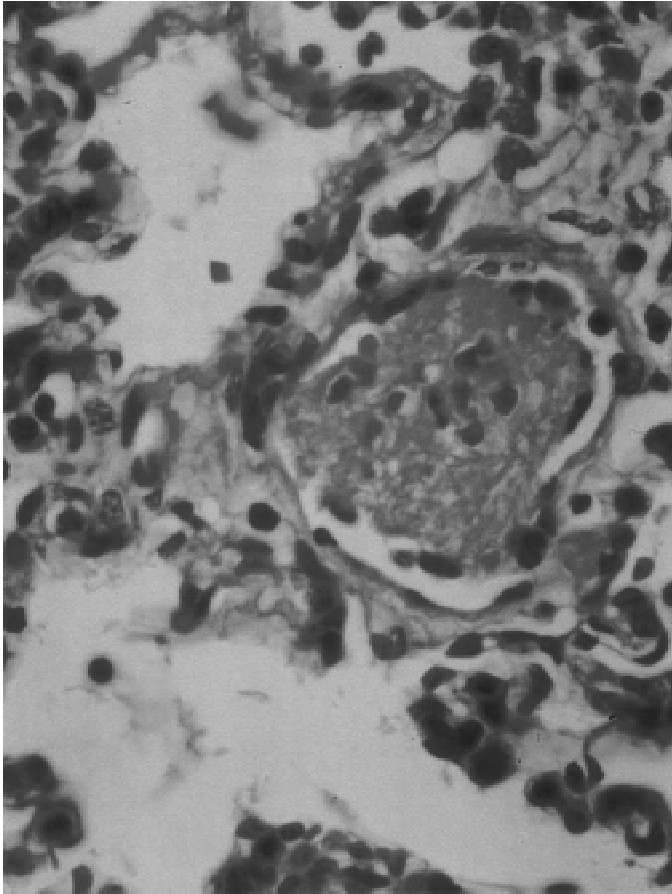


Figure 5: Pulmonary thrombus in treatment animal (magnification $\times 40$).

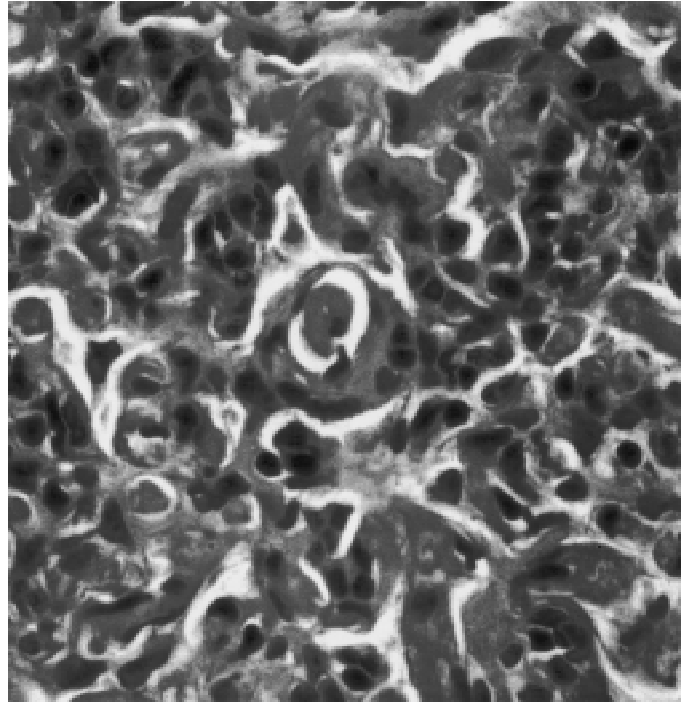


Figure 7: Renal thrombus in treatment animal (magnification $\times 40$).

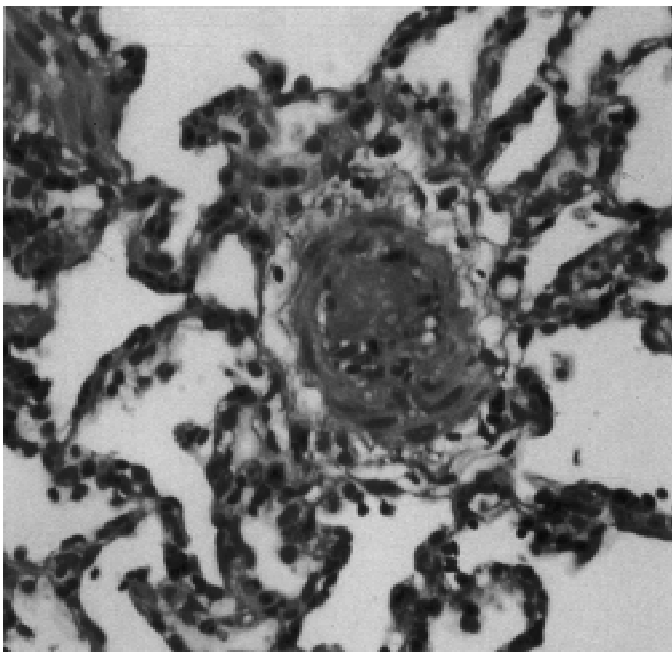


Figure 6: Pulmonary thrombus in control animal (magnification $\times 20$).

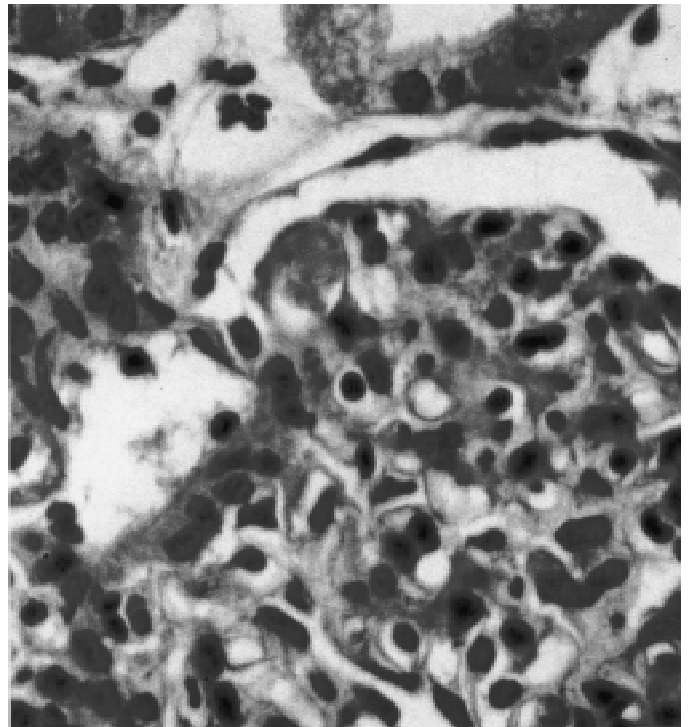


Figure 8: Renal thrombus in control animal (magnification $\times 40$).

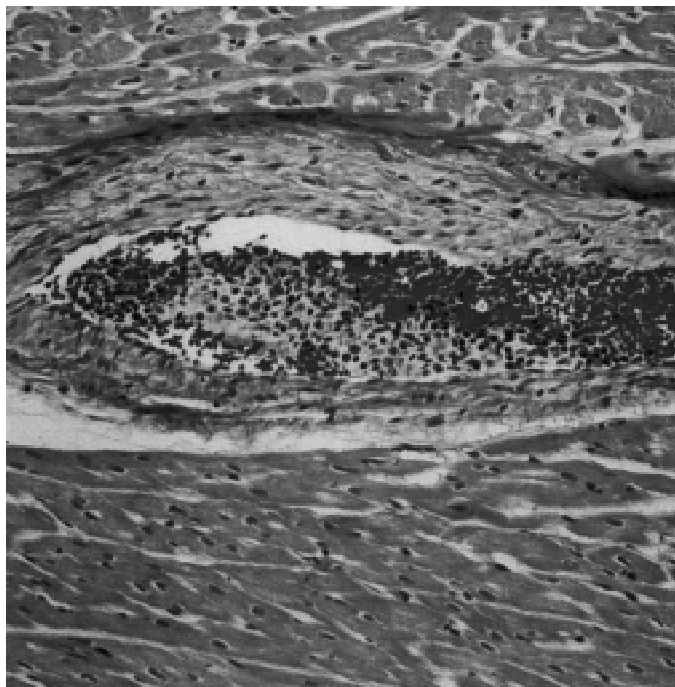


Figure 9: Rare coronary thrombus in control animal (magnification $\times 20$).

surgery (19,20). Circulating levels of tPA increase to approximately 150 % above baseline values during cardiac surgery and activates plasminogen at the site of fibrin formation (21). Therefore, tPA does not induce widespread generalized systemic fibrinolysis, but instead, facilitates clot dissolution at localized sites including surgical vessel trauma.

Fibrinolysis is dependent on conversion of the zymogen plasminogen to the proteolytic enzyme plasmin, which binds to the substrate fibrin, resulting in the solubilization of clot. Activated plasmin has broad specificity and generates polypeptides that digest various coagulation proteins, such as factors I, V, and VIII, fibrinogen, and fibrin. In the process of fibrinogen or fibrin degradation by plasmin, FDPs are generated. CPB has also been shown to reduce PAI, leading to a primary fibrinolytic condition, which increases the potential for postoperative hemorrhage (22). The activation of plasmin further stimulates other serine proteases throughout the coagulation cascade, which compromises postoperative hemostasis (23).

Various studies have reported on differing EACA protocols; however, the ideal dosage of synthetic antifibrinolytic agents remains controversial (24–26). Several dosing schemes are as follows: administration commencing pre-CPB, added to the pump prime, and given at the termination of CPB. A protocol utilizing a higher dose of EACA (30 g total) has been used successfully (27). Eberle and colleagues compared high-dose EACA to aprotinin in first time coronary operations and concluded that, although EACA was effective in inhibiting CPB-induced fibrinolytic activation and postoperative bleeding, aprotinin remained superior over EACA (28).

The antifibrinolytic and procoagulant qualities of aprotinin have been shown to be mediated by inhibition of serine proteases in the blood (16). Aprotinin's antifibrinolytic action is accomplished by the inhibition of the serine protease plasmin, which is where its antifibrinolytic effects are localized. Kallikrein inhibition may also explain some of the clinical effects of aprotinin administration. During CPB, kallikrein is converted from its precursor prekallikrein as the result of the activation of factor XII by the ECC and by damaged endothelial tissue by surgical incision. Consequently, kallikrein is formed in large amounts during CPB, and the effects of kallikrein system activation are multiple (16). Kallikrein causes activation of the intrinsic clotting cascade, has fibrinolytic activity, and plays a role in the release of various substances associated with the inflammatory response. Therefore, during CPB, aprotinin can inhibit activation of the intrinsic coagulation cascade, attenuate fibrinolytic activity, and decreases the impact of the inflammatory response.

Royston et al. developed a pharmacologic approach to reduce bleeding associated with CPB by administering a loading of 2 million units of aprotinin (postintubation), followed by a continuous infusion of 500,000 units h^{-1} (29). Two million units are also added to the CPB prime to maintain plasma concentrations following hemodilution.

Controversy surrounding antifibrinolytic drugs has centered on their potential for promoting pathologic intravascular thrombosis. Case reports, pertaining to EACA have described excessive thrombus formation on pulmonary artery catheters (30). Blood clot obstruction of the left renal collecting system with the use of EACA have also been reported (31). Hypotension with subsequent tubular necrosis and acute renal failure,

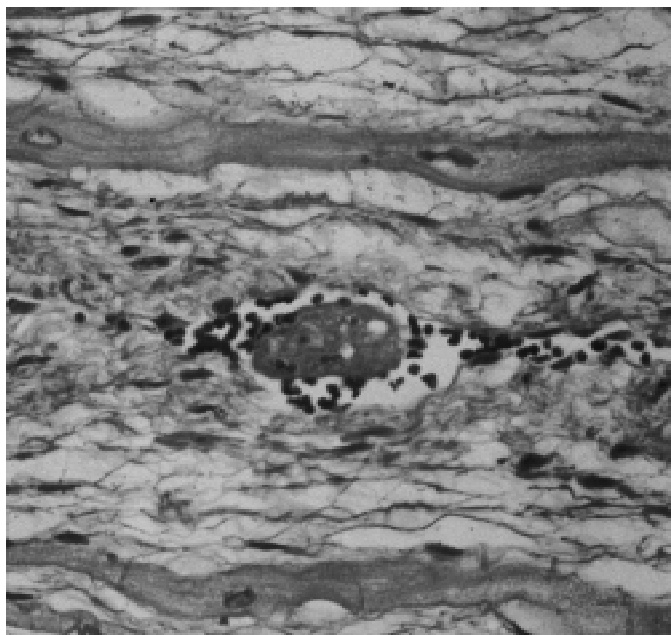


Figure 10: Rare coronary thrombus in treatment animal (magnification $\times 20$).

intraureteral clot formation of hematuria in patients with hemophilia, and diffuse glomerular capillary thrombosis has been reported with the use of EACA (32, 33). Charytan and Purtilo reported a case of glomerular thrombosis and severe hypotension in a patient with prostate carcinoma treated with EACA (34). Renal biopsy before death showed that 75 % of the glomeruli were involved in diffuse thrombosis within the capillary lumen.

Hypercoagulation resulting in the generation of intravascular thrombus has been reported in the literature with the use of aprotinin (35–37). Umbrain and colleagues reported the occurrence of diffuse intracoronary thrombosis in three aprotinin treated patients following protamine reversal of heparin (35). Sundt and colleagues stated that aprotinin should not be given to patients undergoing hypothermic circulatory arrest since these authors observed thrombosis in the postoperative period (36). Early results from the Cleveland Clinic have raised the question as to whether the prophylactic use of aprotinin may increase risk of early graft thrombosis and myocardial infarction in cardiac surgical patients (37).

The concurrent use of aprotinin and EACA has not been extensively investigated, nor do we understand the extent of their combinational effects on patients' hemostasis. There have been reports of the additional use of EACA with aprotinin either resulting in a beneficial or deleterious effect in patient outcome. Cosgrove and associates reported the use of EACA to 4 % of the high-dose and to 2 % of the low-dose aprotinin patients (37). He suggested that improvements in hemostasis for the aprotinin groups may have been attributable to EACA. However, thrombi were found in 6 of 12 vein grafts in 9 patients receiving aprotinin, which may have been contributed to EACA's use. Additional antifibrinolytic therapy in the form of EACA was also reported by Sundt and colleagues (36). EACA was administered to six patients receiving high-dose aprotinin. Three of the six patients developed renal dysfunction and three of whom died. Two of the five patients, who received EACA, showed the presence of intravascular coagulation at autopsy. Identical lesions were also seen in three patients who did not receive EACA. Royston suggests that problems with aprotinin therapy and thrombosis with Sundt and Cosgrove's studies may have been attributed to the additional use of EACA with aprotinin (38). However, whether EACA contributed to intravascular clot formation is unknown, because identical lesions were found in patients receiving no aprotinin in Sundt et al.'s study, and other factors may have contributed to the patients' demise.

In a recent study performed by Levy and associates, 287 patients were randomly assigned to receive either high-dose aprotinin, low-dose aprotinin, pump-prime-only aprotinin, or placebo (39). Overall, the study demonstrated that high- and low-dose aprotinin significantly reduced the requirement for donor blood transfusion in repeat CABG patients. In their discussion, Levy points out that the hemostatic agents, EACA or

desmopressin, were administered because of excessive blood loss in the postoperative period. Unlike the adverse reaction encountered with Sundt and Cosgrove's studies, Levy et al. felt that both of these agents were of potential benefit. The worries and doubts about giving aprotinin to patients remain controversial among cardiovascular surgeons and perfusionists. However, it seems now that the additional use of EACA with aprotinin has stirred a debate. To answer this question better, there is a need to investigate and understand the mechanism underlying the use of aprotinin and EACA concurrently.

TAT is a sensitive marker of thrombin generation. There was thrombin generation during CPB, as demonstrated by the increased levels of TAT after post-sternotomy. Swine in the treatment group demonstrated no significant alterations in the generation of TAT as compared with control. Consistent with the TAT data, no significant alteration in generation of PF 1.2 concentrations were observed during and after CPB. Suppression of fibrinolysis with increasing levels of thrombin formation may alter the hemostatic potential to form thrombus, as evident in thrombi formation in the heart, lung, and kidney of the treatment animals. tPA released from the vascular endothelium is stimulated by a number of factors occurring during CPB. Increasing PAI-1 levels in the treatment group resulted in low tPA concentrations; thereby, reducing the fibrinolytic activity of plasmin. In contrast, low levels of PAI-1 in the control group resulted in higher levels of tPA concentration; thereby, increasing the fibrinolytic activity.

DD levels provide a measure of the amount of fibrin broken down by plasmin during and after CPB. Lower concentrations of DD in the treatment group demonstrated that the co-administration of aprotinin and EACA therapy either reduced plasmin production or inhibited the action of plasmin on fibrin. Although statistical analysis differentiated the two groups at 5 and 30 min postprotamine, a trend toward decreased DD generation appeared in the treatment group. The increased DD levels in the control group may explain why HiTTs were longer versus treatment group. HiTT activates the common pathway of the coagulation cascade, which involve thrombin activation and the conversion of fibrinogen to fibrin. An increase in HiTT may suggest an increased activity of antithrombin III to form TAT and/or decreased levels of fibrinogen. FDP, such as DD promotes further breakdown of fibrinogen and, therefore, result in prolonged HiTT.

Although excessive bleeding is widely recognized as a common complication of cardiac surgery, the recent success of antifibrinolytic drugs as prophylactic hemostatic agents has received little attention outside the surgical literature. The etiology of the coagulopathy following cardiac surgery is clearly multifactorial; however, the success of antifibrinolytic drugs as hemostatic agents suggest that fibrinolysis contributes to bleeding in this setting. Increasingly widespread administration of these drugs necessitates increased awareness of the risks and benefits posed by perioperative antifibrinolytic therapy.

The co-administration of aprotinin and EACA suppressed systemic fibrinolytic activity; however, no significant alterations in the generation of thrombin were detected. Suppression of fibrinolysis in the absence of concomitant reductions in thrombin generation suggests that aprotinin and EACA may potentiate a hypercoagulable prothrombotic state. No difference in treatment and control groups could be observed with regard to thrombin generation, as indicated by TAT and PF 1.2. It is likely that over the next few years, it will be possible to administer a safe and effective prophylaxis to prevent surgical bleeding. In addition, the results obtained from this study will provide a foundation on which further work in human medicine could be stimulated to assess the potential benefit or harm of the co-administration of these agents.

ACKNOWLEDGMENTS

The authors thank the American Society of Extracorporeal Technology for funding a portion of this research. In addition, we thank Gish BioMedical, Medtronic Cardiopulmonary, International Technidyne, and Haemoscope Corporation for their support. Finally, we thank Dr. Stanley J. Radio for his expertise in the field of pathology and Tab W. Burkman for allowing us to utilize his laboratory facilities.

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