

# The Effect of Surface Modification and Aprotinin on Cellular Injury during Simulated Cardiopulmonary Bypass

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**Abstract:** Cardiopulmonary bypass (CPB) elicits derangements to the formed elements of blood because of the physical stresses of extracorporeal flow. Methods of reducing the impact of CPB include circuit surface modification and pharmacological supplementation. The purpose of this study was to examine the effects of aprotinin in combination with surface modification during simulated CPB. Fresh whole bovine blood was used to prime standard CPB circuits divided into four groups ( $N = 3$ ): control (CTR), aprotinin 300 KIU/mL (APR), Poly (2-methoxyethyl-acrylate) coating (PMEA), and APR with PMEA (APR-PMEA). Physical stresses included venous reservoir negative pressure ( $-85$  mmHg), arterial line pressure of 150 mmHg at 5 LPM, and air-blood interface, applied over a 90-minute period. Samples were drawn at the following times: 0, 10, 45, and 90 minutes. Endpoints included platelet count (PLT), plasma-free

hemoglobin (PFHb), and thromboelastography (TEG). PLT did not change ( $138.9 \pm 15.0$  vs.  $102.9 \pm 21.0$ ,  $p = \text{ns}$ ) throughout the 90-minute experimental periods in any group. PFHb increased significantly (mean of 19-fold) throughout the experiment, but was not affected by any treatment. The TEG index declined in the CTR ( $3.6 \pm 0.4$  vs.  $-16.2 \pm 2.9$ ,  $p < .0003$ ), PMEA ( $5.9 \pm 0.8$  vs.  $-2.7 \pm 3.8$ ,  $p < .02$ ), and APR-PMEA ( $4.6 \pm 1.0$  vs.  $-2.8 \pm 0.3$ ,  $p < .0003$ ) groups, but not in the APR group ( $3.6 \pm 2.2$  vs.  $-1.3 \pm 3.3$ ,  $p = .10$ ). In conclusion, neither APR nor PMEA had an effect on either red cell hemolysis or PLT, but APR treatment alone significantly attenuated the derangements in coagulation induced in this extracorporeal model. **Keywords:** aprotinin, cardiopulmonary bypass, biocompatibility, suction, cellular activation. *JECT. 2002;34:267-270*

The application of negative pressure to remove blood from the surgical field has come under increased scrutiny during cardiopulmonary bypass (CPB) and other surgical procedures. Negative pressure has been used in such procedures as autotransfusion, kinetic-assisted venous drainage, vacuum-assisted venous drainage (VAVD), and suction from the field. Concerns have been raised as to the extent of damage to blood attributable to the application of negative pressure (see 1, 3-5).

Previous work by Mullholland et al. has established that the blood-to-air interface and negative pressure are the most damaging, dynamic, nonphysiological forces to the formed elements of blood (1). The trauma induced by these two factors increases in a linear fashion once the threshold value of  $-120$  mmHg is reached. Negative pres-

sure and the air-to-blood interface have been shown to be more damaging than the shear stresses, positive pressures, wall impact forces, and blood-nonendothelial surfaces to which blood is exposed in the extracorporeal circuit (ECC) (1). With the reinfusion of activated blood, the patient's immune response may be activated, causing impairment of cardiac, pulmonary, and renal function as well as cerebral circulation (2). Another concern induced by the collection method is hemostatic derangement effecting platelet adhesion and dysfunction, which promotes coagulopathies (3). Mejak et al. identified coagulopathies as the number one incident leading to mortality in adult CPB (4,5).

Autotransfusion and other blood salvage procedures have been shown to reduce patient blood loss and decrease the need for perioperative blood transfusions from autogenic donor sources (2,6,7). The quality of the reinfused blood products recovered by autotransfusion is dependent on the aforementioned factors involved in the collection and processing sequence (2). Before of increas-

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ing trends toward reduction of perioperative blood loss, it has become necessary to identify ways to reduce the effects of nonphysiological forces.

Research has shown that aprotinin (Bayer Pharmaceuticals, West Haven, CT) decreases the activation of formed elements in blood exposed to excessive nonphysiological forces (6,8–12). In addition, aprotinin can have both pro and anticoagulative effects. Aprotinin aids in coagulation by affecting serine proteases and preserving platelet function (by reducing glycoprotein loss, while in granulocytes, it prevents the proinflammatory adhesive glycoproteins) (5,9,12,13). Although the anticoagulant mechanisms of aprotinin are relatively unknown, it is believed the activation of factor II (prothrombin) and XII (Hagemann's) are inhibited by aprotinin. (6,9,12–14). The addition of aprotinin to the ECC has also been shown to inhibit fibrinolysis by reducing the release of tissue plasminogen activator (5,8,12).

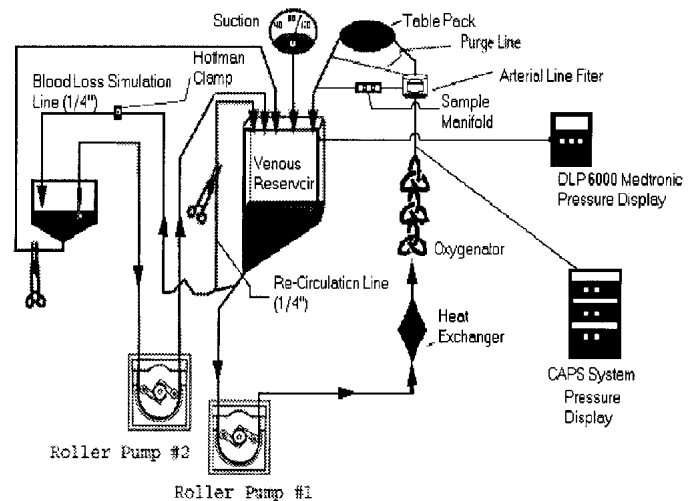
Aprotinin is generally added to the ECC at a standard loading dose of 200 Kallikrein Inhibitor Units (KIU)/mL, which has been shown to have a positive impact on the reduction of activation (5,6,9,10,11,15). Poly (2-methoxyethylacrylate)-coated (PMEA) circuits (Terumo Cardiovascular, Ann Arbor, MI) are growing in use as replacement for traditional noncoated circuits. PMEA has been projected to reduce activation of the formed elements of blood by minimizing contact activation. The purpose of this study was to examine the effects of aprotinin in combination with surface modification (PMEA) during simulated CPB. It is hypothesized that PMEA and aprotinin when used alone or in combination will show no benefit in reducing blood trauma.

## MATERIALS AND METHODS

### Circuit Setup

An in-vitro circuit was designed to mimic normal CPB, as shown in Figure 1. The design specifically incorporated negative pressure and an air-to-blood interface to simulate the clinical situation considered most likely to result in blood activation. The ECC was constructed using roller pump #1, a hard-shell cardiotomy reservoir (simulated chest cavity), and a venous reservoir with vacuum applied negative pressure.

The ECC consisted of a membrane oxygenator (Terumo Cardiovascular, Ann Arbor, MI) and an integrated hard-shell venous reservoir. The arteriovenous loop was composed of 3/8-inch polyvinyl chloride (PVC) with a 1/2-inch boot. A standard bypass 1/4-inch recirculation line was used. A line pressure of  $145 \pm 5$  mmHg was maintained throughout the procedure. Blood loss was simulated by connecting an additional line to the recirculation line (with a y-connector) and incorporating suction of blood from the cardiotomy reservoir (representing the chest cavity).



**Figure 1.** CPB circuit setup showing negative pressure applied to the venous reservoir and an air-to-blood interface in the simulated chest cavity.

A fluted tip suction wand was inserted into the simulated chest cavity, creating an air-to-blood interface. The wand was submerged in the blood to maintain a 1:1 air-to-blood ratio and to mimic suction from the surgical field. Suction was generated using roller pump #2. A 1/4-inch PVC suction line and a one-way, duckbill valve was connected to a filtered port on the venous reservoir. Using a vacuum device, a negative pressure of  $85 \pm 5$  mmHg was applied to the venous reservoir. The negative pressure was applied to represent the accepted upper limit of VAVD.

### Experimental Protocol

Forty liters of fresh, whole bovine blood were collected the morning of each experiment. The blood was anticoagulated with heparin (5 IU/mL) to maintain an activated clotting time (ACT) of  $\geq 480$  seconds. The integrity of the blood was maintained using continuous circulation at 500 mL/min and temperature regulation at  $37 \pm 1^\circ\text{C}$  using a heater/cooler (Terumo Cardiovascular, Ann Arbor, MI). Circuits were flushed with  $\text{CO}_2$  and primed with Plasma-lyte A. A hematocrit of  $23 \pm 2\%$  was maintained throughout the experimental period. Roller pump #1 was set at 5 Lpm and roller pump #2 was maintained at 1 Lpm. The blood loss and line pressure were held constant by adjustment of variable resistant clamps.

A total of 12 experimental circuits randomly divided into four groups ( $N = 3$ ), were run for a period of 90 minutes. The control group was non-PMEA-coated circuitry with no aprotinin added. The second group consisted of non-PMEA circuitry treated with full dose aprotinin (200 KIU/mL). Group three was composed of PMEA circuitry with no aprotinin added, and group four consisted of both PMEA-coated circuitry and aprotinin.

### Laboratory Analysis

Samples were taken at four times throughout each experimental procedure:  $t = 0$ ,  $t = 10$ ,  $t = 45$ , and  $t = 90$  minutes. The negative pressure and air-to-blood interfaces were initiated following collection of the baseline sample. The samples were assayed for plasma-free hemoglobin (PFHb), total protein (TP; refractometer A 300 CL, Atago Co., Tokyo, Japan), activated clotting time (ACT; ACT II, Medtronic Hemotec Inc., Englewood, CO), platelet count (PLT), hematocrit (HCT; Critocaps, Oxford Labware, St. Louis, MO), and thromboelastography (TEG<sup>TM</sup>; Haemoscope 5000, Hemoscope, Skokie, IL).

TEG measurements included R-time (point of initial clot formation), K-time (rate of clot growth), alpha-angle (rate of clot growth), and the maximum amplitude (MA) (strength of clot or direct hemostatic ability). The coagulation index (CI), and over-all measurement of all the above TEG perimeters, was used to determine significance in coagulative difference. PFHb and PLT were performed in the clinical laboratory of the University of Nebraska Medical Center.

### Statistical Analysis

All data were loaded onto a personal computer in a spreadsheet format. Four categories of data were statistically analyzed and compared. These samples include circuits, non-PMEA no aprotinin (control), non-PMEA with aprotinin, PMEA no aprotinin, and PMEA with aprotinin. The three treatment groups allowed two degrees of freedom. A variety of assays were used to determine differences between each circuit including PLT, TEG, and PFHb. Parametric data were analyzed using a two-way analysis of variance. A probability value of less than .05 was considered statistically significant. All data are reported as mean  $\pm$  standard deviation, unless otherwise stated.

## RESULTS

A total of 12 circuits were compared in this analysis. Each of the three experimental groups was compared to the control group. Compared to the control group, there was no significant difference found in the PLT, ACT, HCT, and the TP. Although trends were shown in the various groups, no significant differences were shown in PLT or PFHb. There were significant differences found in the TEG.

### Thromboelastography Change

The R-time was significantly greater in the control group than the other three groups [Control (15.8 $\pm$ 3.3 min), non-PMEA, aprotinin (-1.2 $\pm$ 1.9 min,  $p = .0001$ ), PMEA, nonaprotinin (4.2 $\pm$ 3.3 min,  $p < .0008$ ), PMEA, aprotinin (3.0 $\pm$ 2.2 min,  $p = .0004$ )] (Table 1). The PMEA,

**Table 1.** Mean difference and standard deviations for each PMEA, aprotinin combination.

	X-Coating	Aprotinin	Mean Difference (90 min-0 min)	Standard Deviation
R time	No	No	15.8	3.3
	No	Yes	-1.2	1.9
	Yes	No	4.2	3.3
	Yes	Yes	3.0	2.2
K time	No	No	32.7	6.0
	No	Yes	3.3	1.9
	Yes	No	5.3	5.1
Alpha angle	Yes	Yes	5.7	1.2
	No	No	-48.2	6.4
	No	Yes	-21.9	15.1
MA	Yes	No	-29.5	19.1
	Yes	Yes	-29.5	4.8
	No	No	-39.2	8.0
CI	No	Yes	-24.2	5.3
	Yes	No	-32.2	9.2
	Yes	Yes	-25.3	3.1
CI	No	No	-19.8	3.1
	No	Yes	-4.9	2.0
	Yes	No	-8.5	4.5
	Yes	Yes	-7.4	1.1

nonaprotinin group also showed a significant change (4.2 $\pm$ 3.3 min) as compared to the non-PMEA, aprotinin group (-1.2 $\pm$ 1.9 min,  $p = .042$ ).

In regard to K-time, the PMEA and aprotinin groups were significantly different (5.7 $\pm$ 1.2 min) from the control group (32.7 $\pm$ 6.0 min,  $p = .0001$ ). Each PMEA-protinin combination is shown in Table 1. The difference in the K-time from 90 min to 0 min was significantly greater in the control group than in the other three groups ( $p < .0001$ ). There were no significant differences in alpha angle shown between the control group and the other three groups.

The only significant difference found in the MA was between the control group (-39.2 $\pm$ 8.0 mm) and the non-PMEA, aprotinin group (-24.2 $\pm$ 5.3 mm,  $p < .05$ ). [The CI is a combination measurement of all of the above variables]. The PMEA, aprotinin was significantly different (-7.4 $\pm$ 1.1) from the control group (-19.8 $\pm$ 3.1,  $p < .0010$ ) (Table 1). The difference in CI from 90 min to 0 min was significantly different in the control group as compared to the other three groups [Control (-19.8 $\pm$ 3.1), non-PMEA, aprotinin (-4.9 $\pm$ 2.0,  $p = .0003$ ), PMEA, nonaprotinin (-8.5 $\pm$ 4.5,  $p < .0018$ ), PMEA, aprotinin (-7.4 $\pm$ 1.1,  $p = .0010$ )].

## DISCUSSION

This study was performed to investigate the effects of aprotinin and surface modification (PMEA) on the reduction of activation of the formed elements of whole blood. The TEG results indicated that treatment with aprotinin alone produced significant effects in reducing the damage to blood. Surface modification with PMEA also showed

trends in reducing activation; however no significance was found. Treatment with aprotinin in conjunction with surface modification showed trends in reduction as well. There was no synergism in activation reduction when combining the two, and there was no significance difference in combined reduction of damage.

The simulated CPB circuit, which consisted of damaging, nonphysiological forces, was shown to activate the formed elements of whole blood. These findings supported previous research (1,2,7,8,11). The presence of damaged blood, with potential to be reinfused, supports the need for improved circuit biocompatibility.

The results indicate that both aprotinin and PMEA are effective in reducing activation of whole blood. There is indication that coagulation derangements will also be effectively minimized using these agents during CPB. Previous research has shown aprotinin to be effective in reducing the coagulative defects caused by nonphysiological forces experienced during CPB (8,9,11). Many investigators have reported that aprotinin decreases the need for autogenic blood (6,7,9,10,15–17); while it has also been shown that careful management of the cardiac patient is more effective than pharmacological support in most patients (5).

Both aprotinin and PMEA are forms of surface enhancement used to reduce the degree of contact activation between blood and the ECC. A combination of the two or usage of each individually reduces the damage to whole blood. The results indicate that no synergistic effect occurs between the two; however, each seems effective alone.

Although there were few significant differences in the reduction of activation, there were trends shown in a variety of cases. There is a possibility that many of the trends would have been significant if the limitations of the study had been minimized. Limitations of this study included sample size, duration of simulated CPB, and cost. Each of these variables would have increased the power of the study and might have elicited significant differences. The duration of the simulated CPB or the length of time each circuit was conducted was a limitation because of the amount of activation shown. Certain assays were unable to measure the extreme amount of activation of blood by the circuit arrangement accurately. This further reinforces the fact that large amounts of damage are incurred by negative pressure and an air-to-blood interface during normal CPB.

In conclusion, activation of whole blood was demonstrated using negative pressure and an air-to-blood interface during simulated CPB. The results indicated that

aprotinin and surface modification alone reduced the damaging effects to whole blood. There was no synergistic effect shown between the two. Further study measuring pharmacological maintenance and surface modification should be performed. These studies should have larger sample sizes and should focus on reducing the over-all contact activation caused by blood nonendothelial surfaces.

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