

# The Effects of Aprotinin on Platelet Function in Blood Exposed to Eptifibatide: An *In Vitro* Analysis

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**Abstract:** The preoperative use of platelet inhibitors has increased the risk of bleeding during cardiac surgery. Aprotinin has been shown to preserve hemostatic function in patients undergoing CPB. The purpose of this study was to investigate the effect of aprotinin on coagulation in blood exposed to eptifibatide. Freshly collected bovine blood was used in an *in vitro* model of extracorporeal circulation. Blood was separated into two groups: activated (60 minutes exposure to bubble oxygenation) and nonactivated. Within each group there were four subgroups: control ( $n = 3$ ), eptifibatide ( $2.8 \mu\text{g/mL}$ ,  $n = 3$ ), aprotinin ( $250 \text{ KIU/mL}$ ,  $n = 3$ ), and eptifibatide with aprotinin ( $2.8 \mu\text{g/mL}$ ,  $250 \text{ KIU/mL}$ ,  $n = 3$ ). Twenty-four modified extracorporeal circuits utilizing a hard-shell venous reservoir and cardioplegia heat exchangers were used. Blood flow was maintained at a rate of  $1.25 \text{ L/min}$  for a total of 170 minutes, at  $37 \pm 1^\circ\text{C}$ . Samples

were collected at 0, 20, 50, and 110 minutes with the following variables measured: thromboelastograph (TEG), activated clotting time (ACT), and hematocrit (Hct). Results demonstrated that at 110 minutes, the TEG index (TI) was decreased by four-fold in the activated group compared to the nonactivated group ( $-4.6 \pm 1.2$  vs.  $1.4 \pm 1.5$ ,  $p < .05$ ). The administration of aprotinin resulted in preservation of the TI as compared to eptifibatide-treated blood ( $-4.9 \pm 1.2$  vs.  $-7.9 \pm 1.2$ ,  $p < .05$ ). Aprotinin combined with eptifibatide reduced coagulation derangements when compared to eptifibatide alone ( $-5.2 \pm 1.2$  vs.  $-7.9 \pm 1.2$ ,  $p < .05$ ). In conclusion, aprotinin attenuated the platelet inhibition effect of eptifibatide during *in vitro* CPB, resulting in improved coagulation. **Keywords:** eptifibatide, aprotinin, coagulopathies, platelet dysfunction, cardiopulmonary bypass. *JECT. 2003;35:304-311*

Even with the advances that have been made in anesthetic, surgical, and perfusion techniques, postoperative bleeding is still a major cause of morbidity in cardiac surgery (1,2). In fact, a survey sent by Mejak et al. to all of the major cardiac surgical centers in the United States demonstrated that out of the 141 incidents reported that resulted in death, coagulopathies were responsible for 33% of these. In addition, 315 incidents were reported that resulted in serious injury to patients, out of these, 44% were attributable to coagulopathies (2).

The occlusion of coronary arteries by platelet thrombi is an important pathophysiologic event in acute coronary syndromes (ACS), unstable angina, and myocardial infarctions attributable to ischemic complications of percutaneous interventional (PCI) therapy. The final step of platelet aggregation involves the platelet glycoprotein

(GP) IIb/IIIa. There are now several therapeutic drugs that inhibit the GP IIb/IIIa receptors inhibiting platelet aggregation. One of the most clinically studied GP IIb/IIIa receptor antagonists is eptifibatide (COR Therapeutics, San Francisco, CA). Eptifibatide has been shown to have efficacy in preventing and treating ACS as well as complications attributable to percutaneous transluminal coronary angioplasty (PTCA) (3,4). Although eptifibatide is a relatively safe drug, it has been shown to induce hemorrhage in certain patients, especially those requiring emergency coronary artery bypass graft surgery (4,5).

The process of extracorporeal circulation has been shown to induce many changes to the formed elements of blood, including the derangement of platelet function and derangement of the fibrinolytic pathway (6). There have been many proposed methods to attenuate the negative response to CPB, including the use of biologically active surfaces and pharmacologic therapy. Aprotinin (Bayer Pharmaceuticals, West Haven, CT), a serine protease inhibitor derived from bovine lung tissue, has been shown to

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reduce the incidence of postoperative bleeding and reduce inflammation significantly (6–8). The purpose of this *in vitro* study was to investigate coagulation function in blood activated during simulated CPB, treated with eptifibatid, and the effect of aprotinin.

## METHODS AND MATERIALS

### Experimental Circuit

An *in vitro* analysis was conducted using modified extracorporeal circuits in a model of simulated extracorporeal circulation. Circuits were constructed as either a non-activation circuit (Group I), or an activation circuit (Group II). These circuits were constructed with a recirculation limb, a nonactivation/activation limb, and a treatment limb (Figures 1,2). Fresh, 6 hour, bovine blood was used in all studies, following anticoagulation with 5 IU/mL bovine lung heparin. Blood was diluted with physiologic saline solution to a starting hematocrit of  $25 \pm 2\%$ .

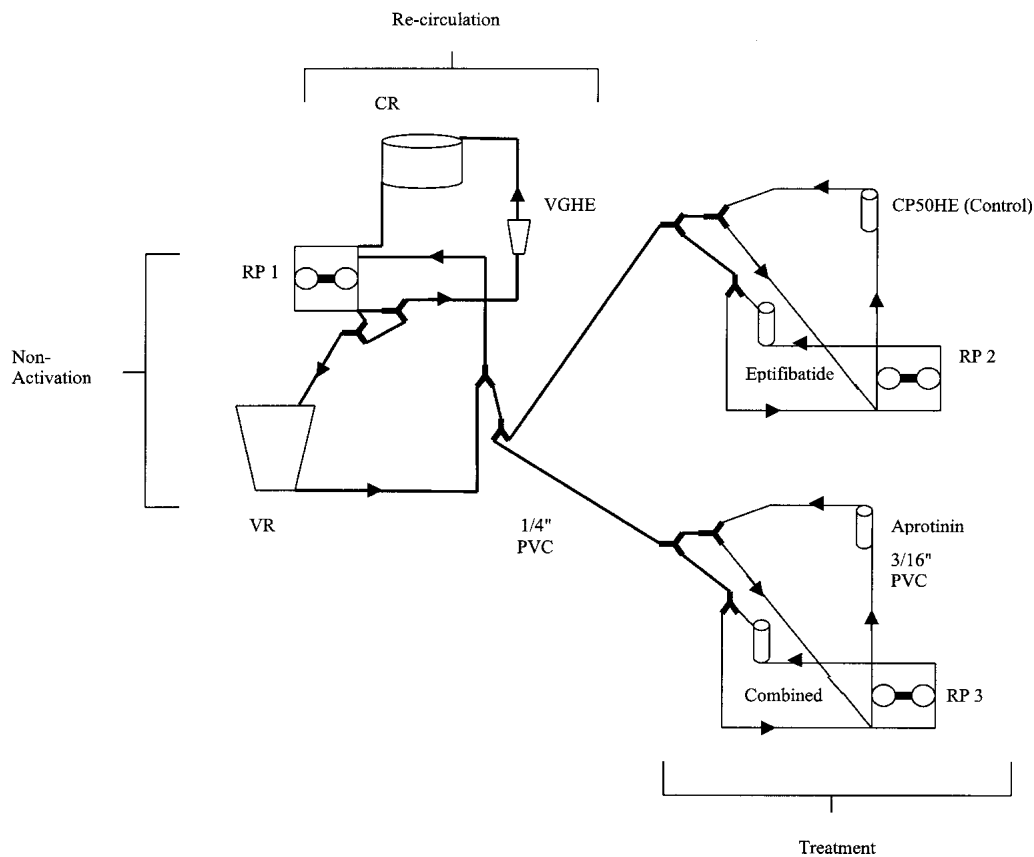
### Recirculation Limb

The recirculation limb consisted of a circuit with a 20-L reservoir in which blood was circulated within using 1/4-inch polyvinyl chloride (PVC) tubing and a twin roller

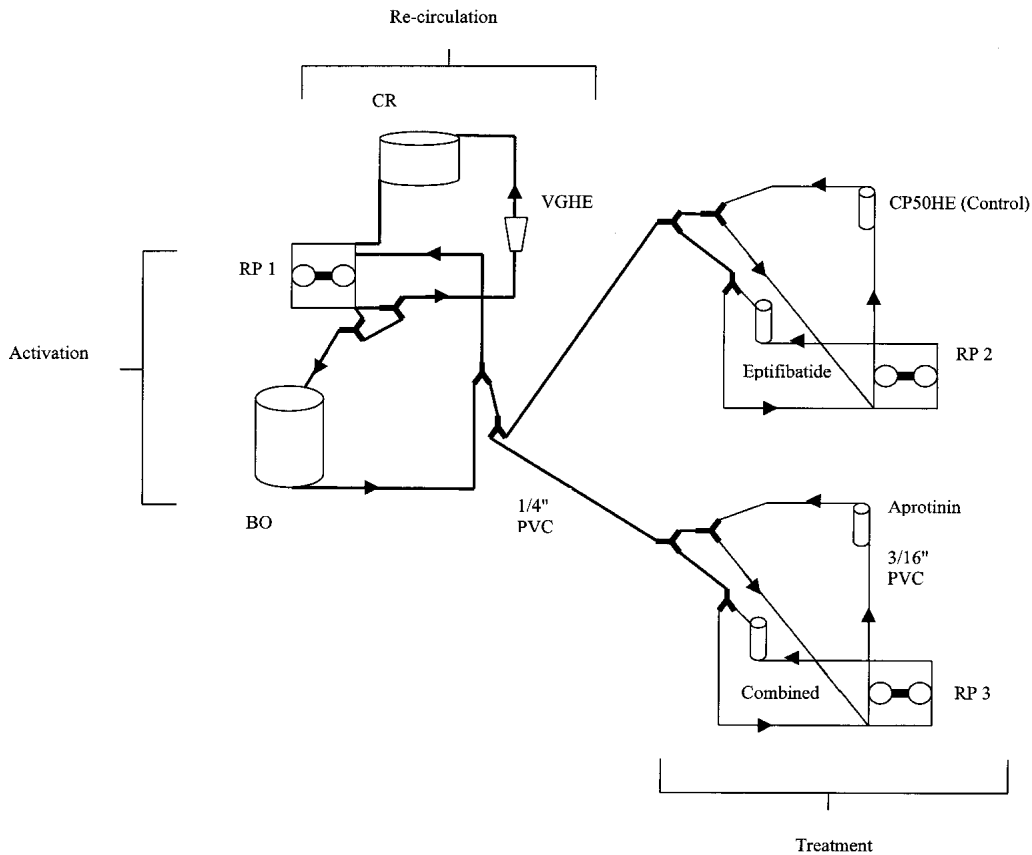
head pump (Stöckert, Munich, Germany). A 1/4-inch Wye connector was placed to direct blood either to the 20-L reservoir for recirculation, or to the activation/non-activation limb of the circuit. This was used to blood prime the activation/nonactivation limb of the circuit. As blood returned to the 20-L reservoir, it passed through a heat exchanger (CSC 14 Cardioplegia Heat Exchanger, Sorin Biomedica, Irvine, CA) to maintain a blood temperature of  $37 \pm 1^\circ\text{C}$ . Blood was circulated through this circuit for the entire duration of each experiment. Both Group I and II included a recirculation limb.

### Activation/Nonactivation Limbs

The activation/nonactivation limbs of the circuit were constructed using an adult bubble oxygenator (Baxter Bos 10, Bentley-Baxter Healthcare Corporation, Irvine, CA) for the activation limb, and the nonactivation stage used a hard-shell venous reservoir (CRF-40 Hardshell Venous Reservoir, Sorin Biomedica, Irvine, CA). Both limbs used 1/4-inch PVC tubing and a twin roller pump (RP 1). The activation circuit used a gas source of 100% oxygen connected to the gas inlet of the bubble oxygenator. Distal to the outlet of the bubble oxygenator/venous reservoir, a Wye connector was placed to allow blood flow to be di-



**Figure 1.** Circuit diagram of the nonactivated phase. RP 1: Roller pump #1, VGHE: Vanguard heat exchanger, CR: Collection reservoir, BO: Bubble oxygenator, 1/4" PVC: 1/4" polyvinyl chloride tubing, 3/16" PVC: 3/16" polyvinyl chloride tubing, CP50HE: Capiiox CP 50 heat exchanger, RP 2: Roller pump #2, RP 3: Roller pump #3.



**Figure 2.** Circuit diagram of the activated phase. RP 1: Roller pump #1, VGHE: Vanguard heat exchanger, CR: Collection reservoir, BO: Bubble oxygenator, 1/4" PVC: 1/4" Polyvinyl chloride tubing, 3/16" PVC: 3/16" polyvinyl chloride tubing, CP50HE: Capiox CP 50 heat exchanger, RP 2: Roller pump #2, RP 3: Roller pump #3.

rected through either the activation/nonactivation limb, or into the treatment limb of the circuit. A luer connector was placed distal to RP 1 to help maintain circuit pressure of 150 mmHg using an aneroid pressure manometer.

### Treatment Limb

The treatment limb of each circuit was composed of identical circuits that facilitated four different experimental treatments to be conducted at the same time. Each of these circuits was constructed with a cardioplegia heat exchanger (Capiox CP 50, Terumo Cardiovascular, Ann Arbor, MI), 1/4- and 3/16-inch PVC tubing, a twin roller pump (Stöckert, Munich, Germany), and a heater-cooler (Dual Heater-Cooler, Terumo Cardiovascular, Ann Arbor, MI). The 1/4-inch PVC tubing was used in priming of each of the individual circuits, while the 3/16-inch PVC tubing allowed for circulation through the cardioplegia heat exchanger.

### Priming Sequence

Both the activation/nonactivation and treatment limbs of the circuit were initially crystalloid-primed with 1-L of balanced physiologic saline solution. When the activation/nonactivation limb was determined to be free of air, the tubing clamps isolating both the treatment limb and the first of the treatment circuits were removed, and a tubing

clamp was placed at the inlet of the oxygenator/venous reservoir. The prime solution was allowed to circulate through the first treatment circuit and de-aired through a port located in the integrated bubble trap of the heat exchanger. Each of the remaining treatment circuits were primed and de-aired in the same manner using tubing clamps to direct the prime solution into each circuit.

### Experimental Protocol

Group I of the experimental protocol (the nonactivation phase) involved circulating the blood through a venous reservoir and heat exchanger for a period of 60 minutes. Group II of the experimental protocol (the activation phase), involved circulating the blood through the bubble oxygenator using a gas source of 100% oxygen for a period of 60 minutes. Blood was circulated at 1.25 LPM in each phase at  $37 \pm 1^\circ\text{C}$ . As soon as the 60-minute period of circulation/activation was completed, the blood was directed into the treatment limb of the circuit. These were blood primed in the same manner that they were crystalloid primed. Blood was directed individually into each circuit with the use of a purge line connected at the top to each heat exchanger to allow the blood to replace the crystalloid solution.

The treatment phase of the experiment included four separate circuits, each of which was assigned to a different

treatment. One circuit served as the control ( $n = 3$ ), to which neither eptifibatide or aprotinin was added. The second circuit was treated with 2.8  $\mu\text{g}/\text{mL}$  eptifibatide ( $n = 3$ ), the third with 250 KIU/mL aprotinin ( $n = 3$ ), and the fourth with 2.8  $\mu\text{g}/\text{mL}$  eptifibatide and 250 KIU/mL aprotinin ( $n = 3$ ). A baseline sample was taken, and then eptifibatide administration occurred immediately after blood was introduced into each of the circuits. After a 5-minute circulation time following eptifibatide administration, the appropriate circuits were treated with aprotinin. Blood was then circulated through all of the circuits at 1.25 L/min and  $37 \pm 1^\circ\text{C}$  for 110 minutes following the addition of aprotinin.

### Sample Analysis

Samples were drawn at four time points: baseline (0), 20, 50, and 110 minutes. Heparinization was reversed with the administration of 1:1 protamine to negate the effect of heparin on the coagulation studies. The samples were then aliquoted into the appropriate volumes for thromboelastography [(TEG) (Haemoscope Corporation, Skokie, IL)], hematocrit, (Hct) and Activated Clotting Time [(ACT)(Hemochron Response, ITC, Edison, NJ)] analysis. TEG parameters measured included R time, K time, alpha angle, maximum amplitude (MA), and TEG index (TI). The R time, also known as the reaction time, represents the time to initial fibrin formation. Normal values range from 7.5 to 15 minutes. The K time, also known as the coagulation time, is the time from the R value until the amplitude of the TEG tracing is 20 mm. Normal values of K time range from 3 to 6 minutes. The alpha angle is the angle that is formed by the up sloping of the TEG tracing from the R-value. Normal values of the alpha angle range between 45 to 50°. Maximum amplitude is representative of the maximal strength of the formed clot, and normal values range between 50 to 60 mm (1). The TI is obtained by discriminate analysis of all the parameters obtained during TEG analysis and enables the clinician to determine normal and abnormal states of coagulation. TI was calculated with the following equation:  $\text{TI} = -7.792 - (0.392 \times \text{R}) - (0.189 - \text{K}) + (0.122 \times \text{MA}) + (0.076 \times \text{Alpha Angle})$ .

### Statistical Analysis

All data were loaded onto a personal computer in a Microsoft Excel spreadsheet format. The data analysis for this paper was performed with Version 8 of the SAS System for Windows®. A mixed effects model was used to examine the effects of treatment and time on the outcome measures with a repeated measures model. This takes into account that measurement over time can be correlated and adjusts standard errors accordingly. Statistical significance was accepted at  $p \leq .05$ . All data are expressed as mean  $\pm$  standard deviation of the mean.

## RESULTS

The determination of adequate activation of the blood in Group II of the experimental protocol was established by comparing the control baseline TI, and ACT raw data values to Group I. TI baseline values were significantly lower in Group II ( $-4.6 \pm 1.2$ ) when compared to Group I ( $1.4 \pm 1.5$ ) ( $p < .05$ ). The control baseline ACT values between Group II ( $138.7 \pm 12.8$ ) and Group I ( $164.0 \pm 18.0$ ) were also significant ( $p < .05$ ).

Analysis of ACT in Group II revealed that there was not a statistical difference between treatment groups. However, there were significant differences in ACT across the different time intervals. Significant differences in ACT values between the treatment groups of Group I were not achieved, although across time, ACT values did significantly vary (Table 1).

TEG analysis of the R time, K time, MA, and alpha angle of Group II revealed that there was a difference, although not significant, between the treatment groups (Table 2). There was a decrease in the alpha angle of the eptifibatide group compared to the control, and an increase of alpha angle in both the aprotinin and combined groups compared to the eptifibatide group. K time was increased in the eptifibatide group compared to the control, and an improvement in K time was seen in both the aprotinin and combined groups compared to the eptifibatide group. MA decreased in the eptifibatide group compared to the control, and an increase of MA was seen in both the aprotinin and combined groups compared to the eptifibatide group. R time was decreased in the eptifibatide group and was lengthened in the aprotinin group. Treatment with eptifibatide significantly decreased TI compared to the control, while treatment with aprotinin reduced the coagulation derangements seen with eptifibatide. Alpha angle and TI were significantly different with time, regardless of treatment (Table 2).

Analysis of the Group I TEG parameters among treatment groups yielded much of the same results, with the

**Table 1.** Activated clotting time (ACT) assessment between the treatment groups and time periods of the activated and non-activated phases (in seconds).

Treatment	Activated	Non-Activated
Control	155.2 $\pm$ 13.0	174.1 $\pm$ 18.0
Eptifibatide	147.2 $\pm$ 13.0	174.7 $\pm$ 18.0
Aprotinin	153.9 $\pm$ 13.0	180.7 $\pm$ 18.0
Combined	163.6 $\pm$ 13.0	191.3 $\pm$ 18.0
	$p = .38$	$p = .28$
Time		
Baseline	138.7 $\pm$ 12.8	164.0 $\pm$ 17.5
20 minutes	141.7 $\pm$ 12.8	174.6 $\pm$ 17.5
50 minutes	167.5 $\pm$ 12.8	192.4 $\pm$ 17.5
110 minutes	172.2 $\pm$ 12.8	189.8 $\pm$ 17.5
	$p = .0004$	$p = .0028$

**Table 2.** Analysis of TEG parameters between the treatment groups and time periods of the activated phase.

Treatment	ANG (°)	TI	K (sec)	MA (mm)	R (sec)
Control	45.7 ± 5.3	-5.8 ± 1.2	4.7 ± 1.2	23.2 ± 3.0	5.7 ± 0.64
Eptifibatide	36.3 ± 5.3	-7.9 ± 1.2	8.0 ± 1.2	19.0 ± 3.0	4.9 ± 0.64
Aprotinin	45.6 ± 5.3	-4.9 ± 1.2	6.2 ± 1.2	23.7 ± 3.0	5.8 ± 0.64
Combined	52.2 ± 5.3	-5.2 ± 1.2	5.9 ± 1.2	21.6 ± 3.0	4.1 ± 0.64
	<i>p</i> = .15	<i>p</i> = .043	<i>p</i> = .28	<i>p</i> = .28	<i>p</i> = .24
Time					
Baseline	29.8 ± 5.8	-8.2 ± 1.2	7.2 ± 1.2	18.3 ± 3.0	6.0 ± 0.50
20 minutes	43.3 ± 5.8	-5.8 ± 1.2	7.4 ± 1.2	25.3 ± 3.0	5.0 ± 0.50
50 minutes	45.1 ± 5.8	-5.1 ± 1.2	5.2 ± 1.2	21.4 ± 3.0	5.1 ± 0.50
110 minutes	61.3 ± 5.8	-4.6 ± 1.2	5.0 ± 1.2	22.5 ± 3.0	4.4 ± 0.50
	<i>p</i> = .0016	<i>p</i> = .039	<i>p</i> = .38	<i>p</i> = .069	<i>p</i> = .12

ANG: the angle formed by the upslope of the TEG tracing from the R value, TI: TEG Index (a calculated number based on R time, K time, MA, and ANG), K: Kinetic time representing rate of clot growth, MA: Maximum amplitude of the TEG tracing, R: Reaction time for initial clot formation.

exceptions being an increase in R time of the eptifibatide group, and significance in TI among the treatment groups was not reached. Statistical significance was reached in alpha angle, R time, and TI with respect to differences in time (Table 3).

It was found that pharmacologic treatment had no significant effect on hematocrit in either Group I or II. However, there was a significant difference found in hematocrit of both groups in respect to time (Figure 3).

## DISCUSSION

Platelet aggregation plays a seminal role in coronary artery thrombus formation and is a primary factor in both acute ischemic coronary syndromes (ACS), and acute ischemic complications associated with PTCA (3–5). During the last two decades, PTCA has become a common approach in treating ischemic heart disease. Even though short-term success rates have been reported to be at least 90%, coronary intervention may still be complicated by

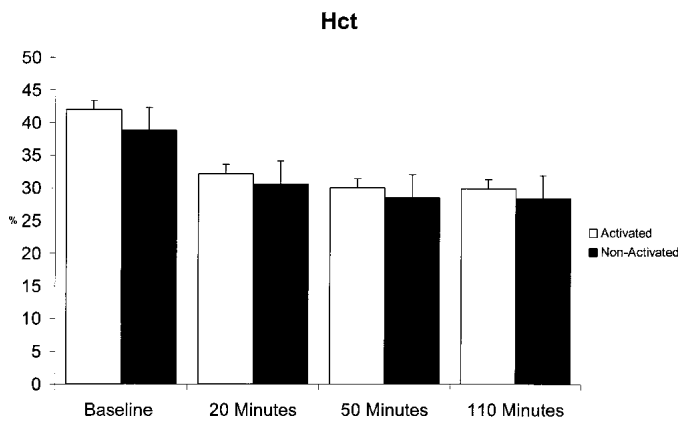
AICS and other complications attributable to PTCA (9). The extent of thrombotic occlusion determines the clinical presentation of AICS, which range from unstable angina to acute myocardial infarction, to sudden ischemic death. Therefore, one of the main goals in patient management of AICS is the inhibition of the key pathologic processes of thrombus formation, which is generally mediated by platelets (3,9).

The final common pathway to platelet aggregation and coronary thrombosis involves the activation of the platelet glycoprotein (GP) IIb/IIIa. GP IIb/IIIa is part of a family of heterodimeric cell surface proteins called integrins that play a role in cell adhesion. GP IIb/IIIa is only found on platelets and other cells that are derived from megakaryocytes. It is also the most common protein on the surface of platelets, numbering as high as 80,000 copies on a single platelet, accounting for 1–2% of the total protein mass of platelets (3). Upon platelet stimulation, GP IIb/IIIa is able to act as a receptor for fibrinogen and von Willebrand's factor. Ligands formed between these molecules with ad-

**Table 3.** Analysis of TEG results between the treatment groups and time periods of the nonactivated phase.

Treatment	ANG (°)	TI	K (sec)	MA (mm)	R (sec)
Control	64.8 ± 6.6	4.2 ± 1.7	2.7 ± 1.4	64.9 ± 4.9	3.1 ± 0.54
Eptifibatide	42.8 ± 6.6	-0.93 ± 1.7	7.8 ± 1.4	50.5 ± 4.9	4.6 ± 0.54
Aprotinin	47.8 ± 6.6	2.9 ± 1.7	5.2 ± 1.4	56.4 ± 4.9	5.0 ± 0.54
Combined	46.9 ± 6.6	-0.97 ± 1.7	6.4 ± 1.4	51.4 ± 4.9	4.8 ± 0.54
	<i>p</i> = .065	<i>p</i> = .056	<i>p</i> = .071	<i>p</i> = .072	<i>p</i> = .075
Time					
Baseline	33.8 ± 6.1	4.9 ± 1.5	6.8 ± 1.4	53.5 ± 4.5	5.5 ± 0.50
20 minutes	53.0 ± 6.1	-0.89 ± 1.5	4.4 ± 1.4	53.3 ± 4.5	3.8 ± 0.50
50 minutes	56.5 ± 6.1	-0.14 ± 1.5	5.7 ± 1.4	56.0 ± 4.5	4.5 ± 0.50
110 minutes	58.9 ± 6.1	1.4 ± 1.5	5.1 ± 1.4	60.4 ± 4.5	3.7 ± 0.50
	<i>p</i> = .011	<i>p</i> = .018	<i>p</i> = .70	<i>p</i> = .51	<i>p</i> = .047

ANG: the angle formed by the upslope of the TEG tracing from the R value, TI: TEG Index (a calculated number based on R time, K time, MA, and ANG), K: Kinetic time representing rate of clot growth, MA: Maximum amplitude of the TEG tracing, R: Reaction time for initial clot formation.



**Figure 3.** Hematocrit assessment (Hct) assessment over time of the activated and nonactivated phases (measured as a percentage).

acent platelets leads to the formation of platelet aggregates and coronary thrombi (3).

Traditionally, the approaches to treating and preventing coronary thrombosis have included the use of antiplatelet therapy with aspirin and anticoagulation therapy with heparin. Although these strategies have been clinically useful, there have been discoveries about the pathophysiology of coronary artery thrombosis that point out limitations of both drugs (3). Because GP IIb/IIIa has been shown to play an important role in coagulation and the fact that it is largely limited to the platelet surface, GP IIb/IIIa inhibition through pharmacological intercession has been studied intensely in the setting of coronary intervention (3).

The benefits of such drugs help treat evolving myocardial ischemia and prevent secondary effects of heart disease (10). One of the most clinically studied GP IIb/IIIa receptor antagonists is eptifibatid and has been shown to have excellent efficacy in preventing and treating ACS as well as complications attributable to PTCA (3,11,12). Eptifibatid, a cyclic heptapeptide, is a competitive inhibitor of the GP IIb/IIIa receptor complex, and has a short onset of action and a short duration of action (13). Eptifibatid has been shown to be effective in reducing the occurrence of death and myocardial dysfunction in patients with non-Q wave myocardial dysfunction, unstable angina, as well as the occurrence of complications during PTCA (4,5).

Although most of the patients treated with eptifibatid tolerate the drug well, there are a number of patients who do not and are at risk for developing bleeding complications, particularly in patients who require emergent cardiac surgery. A recent study conducted by Mejak et al. revealed that the most common perfusion incident occurring after cardiopulmonary bypass (CPB) was coagulopathic in nature and occurred at a rate of 1 out of every 771 procedures (2). Using trial data from the Platelet Glycoprotein IIb/IIIa in Unstable Angina: Receptor Suppres-

sion Using Integrilin Therapy (PURSUIT), it was shown that all patients who received eptifibatid treatment ( $n = 1756$ ) compared to those receiving placebo ( $n = 1766$ ), had a greater incidence of severe (2.1% vs. 1.2%), moderate (15.0% vs. 12.3%), and mild (32.6% vs. 17.7%) ( $p < .001$ ) bleeding according to guidelines set forth by the Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries (GUSTO) trial. Studies have also shown that approximately 15–25% of patients with unstable angina or non-Q-wave MI undergo emergent coronary artery bypass graft (CABG) surgery after treatment with a GP IIb/IIIa inhibitor (5). Because of the problem of possible underlying coagulopathies in these patients, the impact of eptifibatid is relatively unknown where cardiac surgery is necessary. The interval from drug administration to operation has a significant effect on postoperative bleeding and the need for transfusion, with shorter intervals increasing this risk (14). The outcomes of these patients treated with GP IIb/IIIa inhibitors will ultimately depend on the further development of efficacious strategies to control bleeding (4,5).

The activation of certain blood constituents during CPB is understood to cause bleeding problems postoperatively because of the untoward alteration of platelets. These defects induce thrombocytopenia, a decrease in response to aggregating agents, the formation of aggregate emboli, the loss of  $\alpha_2$  adrenergic and fibrinogen receptors, the secretion of thromboxane  $A_2$ , and the release of platelet granule contents (15,16). The reported incidence of postoperative bleeding is between 4 to 32% in patients undergoing cardiac surgery, which increases mortality risk (1). Within seconds of blood contact with the extracorporeal circuit (ECC), plasma proteins, inducing fibrinogen, adhere to the ECC surface. Fibrinogen stimulates platelet adhesion, resulting in the loss of the glycoprotein IIb/IIIa receptors (1).

Aprotinin is a naturally occurring serine protease inhibitor isolated from bovine lung (6–8,17). Aprotinin acts on various serine proteases including kallikrein, plasmin, trypsin, urokinase, elastase, and thrombin while preserving platelet function (6–8,18). The hemostatic effect of aprotinin has been related to protection of platelets, inhibition of the intrinsic coagulation pathway, and prevention of hyperfibrinolysis (15). Aprotinin is able to preserve platelet function through the inhibition of the release of  $\beta$ -thromboglobulin, preservation of adenosine diphosphate and collagen-induced platelet aggregation, as well as the preservation of platelet adhesive receptors (15). The platelet protective effect of aprotinin has been described as being indirect and related to the inhibition of platelet agonists produced during CPB, including reducing the activated components of the complement system and inhibition of neutrophil activation (15). Kawasuji et al. reported that total perioperative blood loss was significantly

less in patients treated with an aprotinin loading dose of 30,000 KIU/kg in the priming volume and 7500 KIU/kg/h intravenously compared to the placebo group ( $803 \pm 216$  vs.  $1277 \pm 627$  mL) (16).

Analysis of ACT results revealed that clotting time increased significantly in both the activated and nonactivated groups in respect to time. However, there was no significance reached in ACT values between the different treatment groups. The increase in ACT seems to be more likely caused by the loss of factors in the coagulation cascade or thrombocytopenia caused by insufficient platelet factor 3 caused by the interaction of the blood with the foreign surface of the extracorporeal circuit rather than the administration of pharmacologic agents.

The TEG has been shown to be 80–100% accurate in predicting post-operative bleeding and is more reliable than ACT monitoring. The endpoint of ACT analysis is the formation of fibrin, thus, little information can be gained about platelet function. The TEG has an advantage over other coagulation tests in that it is able to assess heparin and platelet–protein interactions and assess coagulation factor and fibrinogen activity. In addition, because TEG analysis utilizes a sample of whole blood, it is able to provide information about the interaction of all plasma proteins and cellular components of the coagulation process (19). Studies have shown that treatment with eptifibatide affects the final pathway of platelet aggregation by blocking the glycoprotein IIb/IIIa receptor that binds circulating fibrinogen or von Willebrand's factor (12). With K time and alpha angle both representing the kinetics of clot formation, fibrin cross linking, and platelet–fibrin interaction, and MA describing the strength of the clot; the expected K value would be increased, alpha angle decreased, and MA decreased with the administration of eptifibatide, resulting in overall decreased coagulation function (TI) (1).

Analysis of TEG results indicate that coagulation function was decreased in the eptifibatide group of Group II, while it was preserved in the aprotinin group. The co-administration of eptifibatide and aprotinin also yielded a preservation of coagulation function compared to the eptifibatide group alone. This same trend was seen in Group I. Although not reaching significance, there was a difference regarding K time, alpha angle, and MA between the eptifibatide group and control group of both Group I and II. This was most significantly demonstrated in the analysis of TI, which demonstrated a significant difference in coagulation function in Group II and a difference approaching significance in Group I.

Although *in vitro* coagulation models may be criticized for their inability to mimic the bioactivity of the intact body, their importance has been proved in many publications (20–22). As such, they should be used as an adjunct with them. This limitation is relevant because in the intact

human body, eptifibatide has a relatively short half-life of 2 hours. However, with the possibility of underlying coagulopathies in addition to the administration of eptifibatide, it may be of clinical value to be able to protect platelet function in a short amount of time in the event of emergent cardiac surgery. The use of bovine blood may have also been a limiting factor in our study; however, it was found that the effect of eptifibatide and aprotinin administered alone was comparable to the effects found in human models.

In conclusion, it was found in this study that the derangements in coagulation seen with the administration of glycoprotein IIb/IIIa inhibitors, including eptifibatide could be ameliorated with the administration of aprotinin. Further *in vivo* studies should be conducted to confirm these results in patients who present to the cardiac operating suite for emergent surgery with glycoprotein IIb/IIIa inhibitors on board.

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