

Original Article

Thrombelastograph Analysis After Heparin Neutralization with Protamine and Heparinase During Cardiopulmonary Bypass

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ABSTRACT

Hemostatic disorders generated during cardiopulmonary bypass (CPB) result in post-surgical hemorrhage, a major cause of morbidity in cardiac patients. Thrombelastography (TEG) monitors elastokinetic whole blood coagulation but is sensitive to low quantities of circulating heparin, obviating its use during CPB. Heparinase is an enzyme that degrades heparin without affecting coagulation proteins that may aid in identifying coagulopathies during CPB.

In the present study two methods of neutralizing heparin were used to generate TEG profiles during CPB. These profiles were generated by adding 0.03 ml protamine (0.0134 mg) or heparinase (6 U) 0.33 ml samples of heparinized blood. Samples were drawn pre-CPB, 10 and 30 minutes after initiation of CPB, 5 minutes after administration of protamine and 10 minutes after administration of platelet-rich-plasma, from 11 patients undergoing cardiac surgery. The results indicate a significantly higher baseline reaction time between pre-CPB in both groups when compared to all other time points ($p < 0.007$). During CPB, both protamine and heparinase reversed the anticoagulant effect of heparin, permitting the generation of useful TEG data. We therefore conclude that intraoperative TEG profiles can be produced during CPB for the purpose of identifying hemostatic conditions prior to termination of extracorporeal circulation.

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INTRODUCTION

The morbidity and mortality of patients undergoing cardiac surgery has decreased with standardization of surgical techniques and a greater understanding of myocardial protection. However, postoperative bleeding and coagulopathies continue to be major causes of morbidity in this surgical population. Even though coagulation may be normal preoperatively, the initiation of cardiopulmonary bypass (CPB) induces many events that alter hemostasis. Such events would include: heparinization, hemodilution, platelet sequestration, and cellular trauma (1). These episodes will result in thrombocytopenia, decreased clot retraction, accelerated fibrinolysis and prolonged bleeding time, prothrombin time (PT) and partial thromboplastin time (PTT) (2). Thus, post-CPB hemorrhagic anomalies have been difficult to characterize due to the complexities of hemostasis and the variety of system trauma. Routinely, monitoring coagulation and heparin therapy during and after CPB is done using activated clotting time (ACT) or other available clotting screens. Frequently these tests provide non-specific information about current coagulation status, while normalization of the ACT does not preclude a major coagulopathy. Thrombelastography (TEG) is an available technology that provides whole blood analysis for coagulation monitoring. This process produces a complete profile based on the interaction of the hemostatic complex providing information that characterizes the rate of clot formation, clot strength and clot dissolution (3).

TEG^a is currently used as a monitor of coagulation during liver transplantation (4,5). More recently, TEG has been used as an indicator for post-CPB coagulopathies (6,7), where it has been demonstrated that TEG could predict, with 100% accuracy, problems leading to postoperative hemorrhage and the need for reoperation. This simple and easy to use technology was developed and described by Hartert in 1948 (3). A sample of whole blood or platelet-rich-plasma (PRP) is placed into an oscillating cup which has been preheated to 37°C. A plastic pin is suspended by a torsion wire in the sample and the oscillating motion is transferred to the pin as fibrin strands form between the sides and bottom of the sample cup and pin. Thus, the TEG coagulation analyzer^a monitors and records the shear elasticity changes of whole blood or platelet-rich-plasma. The coagulation profile produced is a measure of the kinetics of clot formation, strength and dissolution. Being a whole blood analysis, it is sensitive to all interacting cellular and plasmic factors in the blood. The resulting profile can be used to interpret the hypo-, normal and hypercoagulable state of the sample and its degree of lysis.

At present, TEG profiles can only be obtained pre- and post-CPB because heparinization makes the meaningful evaluation of platelet function and overall hemostasis difficult. Activated clotting time with protamine titration can be used in the presence of heparin to monitor the onset of coagulation (8).

However, ACT alone will not adequately monitor the strength and stability of the clot that is formed. This data must be known to accurately manage postoperative hemorrhage. TEG

can be used to monitor coagulation status (9) and postoperative coagulopathies (6,7). However, even modest doses of heparin greatly prolong the onset of coagulation, thus preventing the rest of the TEG profile from developing within a clinically useful period of time. The purpose of this investigation is to develop and test a TEG analysis procedure modified to eliminate the overriding effects of heparin. Two methods are presented which effectively negate the effect of heparin, thereby allowing the benefit of TEG analysis during CPB.

MATERIALS AND METHODS

In this Internal Review Board approved study, blood samples were collected from 9 men and 2 women (n = 11) presenting for cardiac surgery at the University of Nebraska Medical Center, from June through August 1992. Ages ranged from 46 to 76 with an average age of 62 ± 7.9 years. All patients were treated according to standard anesthetic and surgical techniques for CABG (n = 9) or valvular repair (n = 2). Mean CPB time was 67.4 ± 28.4 minutes with aortic cross-clamp time of 53.6 ± 15.0 minutes. A centrifugal pump^b was used in conjunction with a hollow-fiber oxygenator^c and a closed venous system. The patients were randomly chosen for inclusion into this study.

THROMBELASTOGRAPHY

Standard parameters of coagulation that can be measured on a TEG profile are reflected in the reaction time (R time) which is measured from the time the blood is placed in the cup until an amplitude of 2 mm is reached. The R time correlates with the time necessary for initial fibrin formation. The kinetics time (K time) is the interval from the end of the R time until the amplitude of the TEG tracing reaches 20 mm. This time measures the rate of fibrin formation and cross-linking. R+K correlates with the whole blood clotting time. The maximum amplitude (MA) reflects the absolute strength of the fibrin clot and is dependent on platelet number and function. The alpha angle (AA) value measures the slope of divergence of the tracing from the point of the R time. The AA also demonstrates the rate of fibrin clot formation.

General blood and coagulation profiles performed upon admission included: hemoglobin, hematocrit (Hct), PT, PTT, and platelet counts (PLT). Once in the operating room, the patient had baseline ACTs and TEG profiles run. Blood samples were drawn at five different time points during the surgical procedure for TEG profiles: 1) pre-CPB, 2) 10 min after initiation of CPB, 3) 30 min after initiation of CPB, 4) 5 min post-protamine, and 5) 10 min after the autologous PRP was infused. All samples were activated with 1% celite (10) and analyzed within 4 to 6 minutes after phlebotomy. Samples 1, 4 and 5 were assayed directly by allocating 0.360 ml of activated blood into pre-warmed, dispos-

a Thrombelastograph 3000, Haemoscope Corp., Morton Grove, IL 60053

b Medtronic Biomedicus, Eden Prairie, MN 55344

c Univox, Bentley Laboratories, Irvine, CA 92714

able cups and pins. For samples 2 and 3, 0.33 ml aliquots of activated blood were pipetted into the disposable cups and 0.03 ml of either 6 U heparinase^d or 0.0134 mg protamine^e was added to the sample. Care was taken to mix the samples by raising and lowering the TEG pin at least 5 times before layering with mineral oil to prevent sample desiccation. The overall coagulative status of the patient was assessed using the measured parameters of the TEG and a discriminant analysis equation providing a TEG Index (TI). The TI was calculated from the measured parameters from the celite-activated whole blood TEG profiles as $TI = -(0.3258)Rc - (0.1886)KC + (0.1224)Ma + (0.0759)AAc - 7.7922$. The TI is based on a linear combination of weighted TEG variables (10).

Pilot studies were performed prior to initiation of the approved protocol to determine a dose of heparinase or protamine that would neutralize the effect of heparin spiked into normal whole blood at 2 U/ml (i.e., at the lowest clinically effective level of heparinization (11)). Our criteria for heparin neutralization was to maintain TEG R times within the normal range for nonheparinized whole blood samples. In this study, 3 U of heparinase almost neutralized the effect of heparin, the R time was decreased to 26 mm (normal R time, 21-30 mm). In a parallel study with this optimal concentration of heparinase, progressively higher concentrations of heparin, bracketing the clinically effective range, produced increasingly longer R times. The data from this study indicated a greater concentration of heparinase was necessary to neutralize heparin at the upper end of the clinical range. A concentration of 6 U was selected and tested in the last of our preliminary trials. TEG analysis was performed on several noncardiac patients (n = 4), with and without heparinase in the absence of heparin, to insure that heparinase had no effects on TEG parameters. Non-heparinase baseline R time mean was 35.66 mm and the heparinase baseline mean was 36.66 mm. This assay was completed on hypothermic samples. The in vitro protamine dose was determined in a similar manner. Because of concerns of induced anticoagulation with a high protamine concentration, TEG R times were evaluated in a study in which the optimal protamine dose was altered geometrically, while the heparin dose remained constant. R times were lengthened with a dose 4 times greater than that used in the study; thus insuring a dose that did not overcompensate.

Data was analyzed using one-way and two-way analysis of variance. When significant f ratios were reached, Fisher's protected least significance difference was performed. Statistical significance was accepted at the $p < 0.05$ level. All data are represented as mean \pm standard deviation of the mean.

RESULTS

Five patients were in the protamine group and six in the heparinase group. The results of patient preoperative and post-

operative coagulation screens, Hct and ACTs are shown in Table 1.

There were no significant differences between groups preoperatively. There was a decrease in platelet number postoperatively within the protamine group, $p < 0.005$ (Figure 1) and heparinase group, $p < 0.03$ (Figure 2). This is expected as hemodilution and platelet sequestration occur during CPB. Preoperative ACTs were slightly higher than the reported population average (12) (Figure 3) with 154 and 172 seconds for protamine and heparinase respectively. However, these values fit our cardiac surgery patients who often come to the operating room on anti-coagulative therapy. There was no significant difference between the two groups' ACT values ($p < 0.067$). Other pre- and postoperative values show similarity between groups except in PTT (protamine 28.9 seconds and heparinase 43.8 seconds). As with the ACT, the higher numbers can be explained by two patients in the heparinase group coming to the operating room receiving heparin therapy (Table 1). There was no significant difference in PTT ($p < 0.164$).

There was also no significant difference between the two groups' baseline R times ($p < 0.3$). However, there was a statistically significant difference within groups when comparing pre-CPB R times to other collection points. In the protamine group (Figure 4), the pre-CPB R time was statistically higher when compared to the other collection points ($p < 0.05$). The exception was in the post-CPB five minute post-protamine group where no significant difference occurred. In the heparinase group (Figure 5), all collection point R times were significantly less ($p < 0.02$) when compared to the pre-CPB baseline. MA and AA were not different between groups or within groups. The TEG Index showed no inter- or intra-group differences.

DISCUSSION

TEG analysis has been shown to be a pertinent guide in monitoring hypercoagulability, platelet function and fibrinolysis in hypercoagulable patients (13) and those undergoing non-cardiac surgical procedures (14). These authors stated that TEG parameters correlated with overall clot stiffness (platelet count and fibrinogen concentration) and early clot formation dynamics. Recently, other investigators have found TEG monitoring to be a reliable predictive indicator of post-surgical hemorrhage. Spiess, et al. (6), demonstrated an 85% accuracy compared to standard coagulation profiles (51%) and ACTs (30%). Martin (7) predicted with 100% accuracy those patients who developed postoperative bleeding, although he had a 27% false positive rate. A high correlation of predictability was achieved between 24 hour blood loss and platelet number, with the MA from a fifteen minute post-protamine TEG. Spiess showed a linear correlation between TEG variables R+K and PTT (6), while Tuman correlated a lengthy R+K time with near normal MA, as a factor deficiency (9). The predictive accuracy of TEG beyond that of ACTs or coagulation screenings is evident because TEG evaluates the coagulation process from formation to resolution (fibrin-

d Sigma Chemical Co., St. Louis, MO 63178

e Elkins-Sinn Inc., Cherry Hill, NJ 08003

Figure 1
Pre- and postoperative platelets in protamine group.

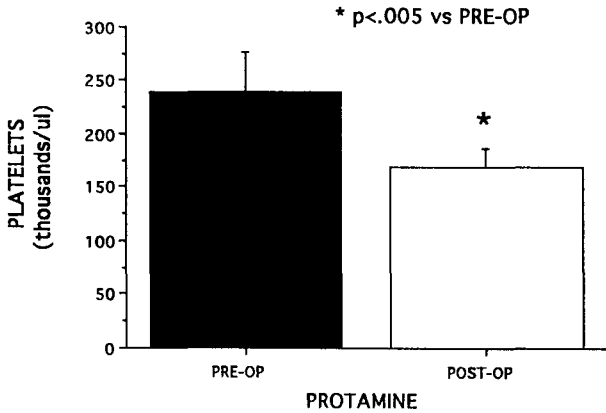


Figure 3
Activated clotting time (protamine vs. heparinase).
ACT=Activated Clotting Time.

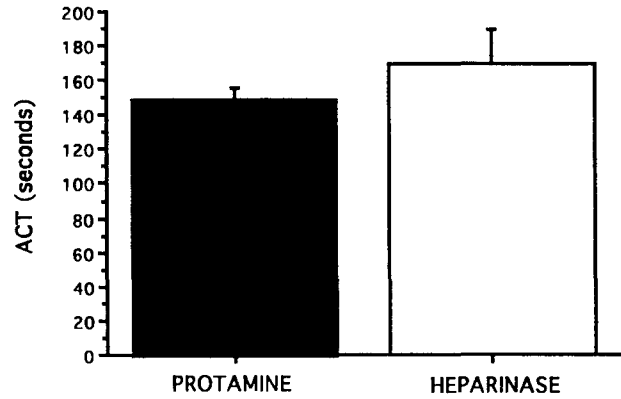


Figure 2
Pre- and postoperative platelets in heparinase group.

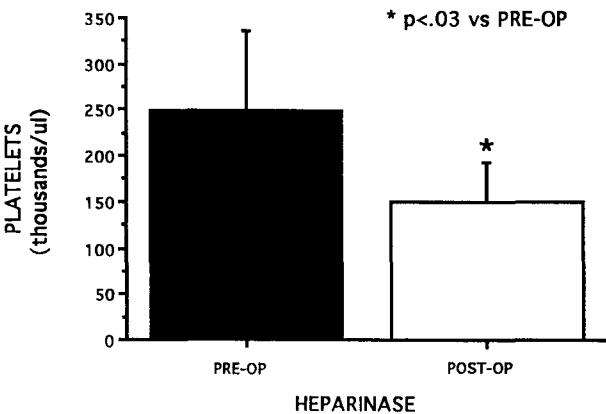
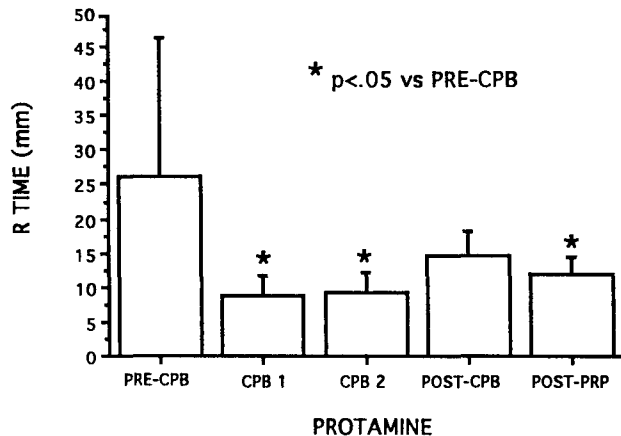


Figure 4
Thrombelastograph R times in protamine group.
CPB=Cardiopulmonary bypass. PRP=Platelet-rich-plasma.



olysis), and considers all interactions between whole blood coagulation participants. Most coagulation screens look at plasma constituents while ACTs can be affected by hypothermia, hemodilution, or factor deficiency.

The use of TEG monitoring has proven beneficial during liver transplantation (4,5) and in predicting postoperative coagulopathies following CPB (6,7,9,13). However, systemic anticoagulation with heparin has precluded measurement of whole blood coagulation with TEG during CPB as the dosage range of heparin required for CPB yields a non-predictive TEG

tracing. Thus, results for TEG profiles have not been available as a guide for coagulation factor therapy during the bypass period. It has been suggested that modification of a TEG blood sample might involve the addition of protamine to determine the presence of heparin in a patient's blood sample. The manufacturer recommends a protamine concentration of 0.0144 mg per ml (10). However, protamine can also be added to a CPB sample to determine preoperative analysis of coagulation conditions. In this study we used a protamine concentration of 0.0134 mg per ml to neutralize the anticoagulant effects of heparin. As the R times

Table 1

Patient Pre- and Postoperative Coagulation Screen

ACT=Activated Clotting Time. Hct=Hematocrit. Hgb=Hemoglobin. PLTS=Platelets. PT=Prothrombin time. PTT=Partial thromboplastin time.

	Protamine		Heparinase	
	PRE-OP	POST-OP	PRE-OP	POST-OP
Hgb	13.9 ± 1.9	10.5 ± 1.63	13.6 ± 1.9	10.8 ± 0.4
Hct	40.9 ± 5.4	30.8 ± 5.2	40.6 ± 5.5	32.4 ± 1.6
PT	11.9 ± 0.5	13.1 ± 1.5	12.2 ± 1.1	12.3 ± 0.2
PTT	28.9 ± 2.4	28.6 ± 4.3	43.8 ± 19.8	27.0 ± 1.4
PLTS	239 ± 33	169 ± 16*	249 ± 79	150 ± 38**
ACT	154 ± 14	145 ± 14	172 ± 2	146 ± 15

*p < 0.005 and **p < 0.03 vs. PRE-OP

All data as MEAN ± SDEV

Table 2

Thrombelastograph Index

CPB=Cardiopulmonary bypass. Post-Prot=Post-protamine. Post-PRP=Post Platelet-rich-plasma.

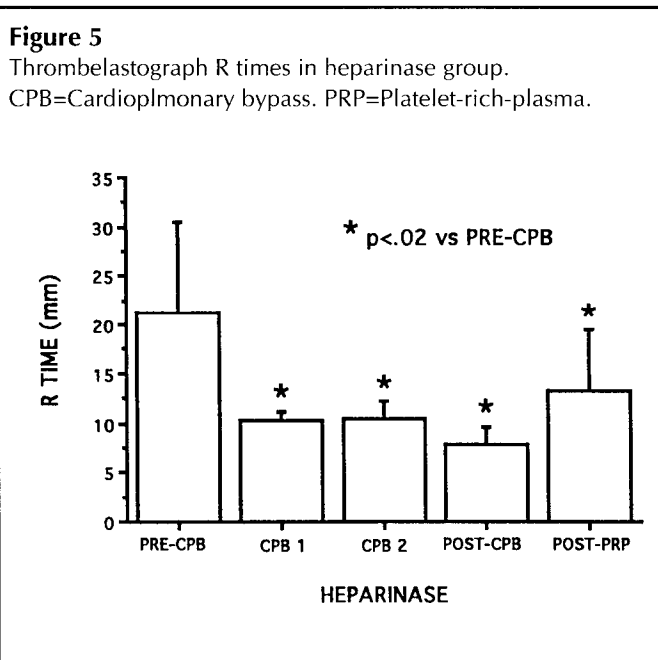
	TEG Index	
	Protamine	Heparinase
PRE-CPB	-0.87 ± 4.50	1.66 ± 1.42
CPB	0.18 ± 2.90	2.29 ± 0.76
CPB 30	1.08 ± 2.42	1.88 ± 0.71
POST-PROT 5	1.20 ± 1.25	2.80 ± 0.65
POST-PRP10	1.47 ± 0.84	3.07 ± 0.76

All data as MEAN ± SDEV

were shortened when protamine was added to heparinized blood samples collected during CPB, it was demonstrated that the heparin was neutralized without a protamine anticoagulant rebound.

Heparinase is an enzyme obtained from *Flavobacterium heparinum* that has been used to normalize the PT and PTT of plasma known to contain heparin (16). This same enzyme has also been used to enzymatically neutralize the anticoagulant properties of heparin in order to obtain baseline clotting times by standard ACT assays (17). Heparinase catalyzes the mucopolysaccharide heparin molecule by cleaving off disaccharides. These disaccharide byproducts are ineffective as coagulation inhibitors and the destruction of heparin (in this manner) is "achieved without any demonstrable deleterious effect upon the plasma coagulation proteins" (16). Heparin reversal was achieved with 6 units/ml heparinase as demonstrated by the significantly shortened R times when CPB and post-CPB samples were compared to baseline values. A similar study has been reported using 5 units/ml heparinase (18).

In this study, the TEG Index (Table 2) remained in the



normal coagulative range (2.0 to -2.0) throughout the surgical procedure. This demonstrates that meaningful profiles were being compiled. If the heparin was not neutralized in either group, the indices would have become less than -2.0 indicating a hypocoagulability.

In conclusion, it has been demonstrated that the use of TEG profiles can be generated by reversing heparinized blood samples in vitro during cardiopulmonary bypass. The TEG profiles during bypass were generally normal in this study. However the thrombelastograph can evaluate platelet function, the intrinsic coagulation pathway and clot lysis to predict which clotting factors may be needed following extracorporeal circulation to treat coagulopathies.

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