

## Original Article

# ***Comparison of Celite and Kaolin Based Heparin and Protamine Dosing Assays During Cardiac Surgery: The In Vitro Effect of Aprotinin***

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### **ABSTRACT**

Both aprotinin and *in vitro* heparin and protamine dosing assays (RxDx System) have been shown to reduce postoperative bleeding in the cardiac surgery patient. While celite activated clotting time tests in the presence of heparin are artificially prolonged by aprotinin, the kaolin based ACT is not. In order to optimize heparin anticoagulation management during cardiac surgery when aprotinin is used, we have evaluated the utility of kaolin based heparin and protamine dosing assays in the presence of aprotinin. *In vitro* studies were performed using normal donor blood after the addition of heparin and/or aprotinin. Clinical evaluations compared anticoagulation management based upon the two activators (celite and kaolin) in 21 patients requiring cardiopulmonary bypass (CPB) who were not receiving aprotinin.

The kaolin heparin and protamine assays were unaffected by aprotinin using *in vitro* aprotinin supplemented normal donor blood samples. The amounts of aprotinin used were equivalent to the calculated plasma concentration in an average size patient (6 liter blood volume) receiving either full (1H) or half (1/2 H) Hammersmith doses. Heparin and protamine doses, based on the kaolin assays, were not significantly different between aprotinin (apr) treated and control samples ( $p > 0.20$ ).

In a clinical evaluation of CPB patients, the C-ACT and K-ACT showed good correlation ( $r = 0.97$ ,  $n = 213$ ), as did the respective dose determination based on the celite and kaolin RxDx assays: heparin dose ( $r = 0.88$ ,  $n = 69$ ) and protamine dose ( $r = 0.88$ ,  $n = 19$ ). These studies illustrate comparable heparin and protamine dosing using either celite or kaolin based RxDx assays during cardiac surgery. These findings also suggest that the kaolin based ACT and dosing assays may be useful in monitoring heparin anticoagulation in cardiac surgery patients receiving aprotinin with the potential of combining these two blood conservation methods.

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## INTRODUCTION

The RxDx<sup>a</sup> hemostasis management system, which consists of *in vitro* dose-response assays and matched pharmaceutical preparations of sodium heparin and protamine sulfate, has been shown to yield optimum anticoagulation management resulting in decreased postoperative blood loss (1) and reduced intra and postoperative blood product usage (1,2). The Heparin Response Test (HRT) quantifies the amount of heparin required to achieve a target level of anticoagulation. The Protamine Response Test (PRT) quantifies the amount of protamine required to return the patient's activated clotting time (ACT) to baseline following cardiopulmonary bypass (CPB) surgery. These assays employ a celite ACT and are performed in conjunction with a standard celite ACT (C-ACT) test to monitor anticoagulation status.

Aprotinin has recently been approved by the FDA as an intraoperative measure to decrease blood loss after cardiac surgery. It is known to artificially prolong the celite based ACT in the presence of heparin (3) and consequently, the celite based RxDx system. A modified RxDx system, using a kaolin based ACT (K-ACT) and kaolin based HRT and PRT tubes has been developed. The kaolin replaces the diatomaceous earth activator found in the C-ACT significantly reducing the artificial elevation of celite ACT clotting times associated with aprotinin use (4,5).

This study compares the celite and kaolin based dosing systems and evaluates the utility of the kaolin RxDx for patients receiving aprotinin. Using a two part design, *in vitro* laboratory analyses were conducted to evaluate the aprotinin effect on ACT clotting times using both celite and kaolin activators as well as the effect on heparin and protamine dose quantification using the RxDx system. Clinical studies were employed to compare the celite and kaolin activated RxDx systems for heparin and protamine dosing in patients undergoing cardiac surgery and requiring cardiopulmonary bypass.

## MATERIALS AND METHODS

### LABORATORY STUDIES

#### Clotting Time Studies

Celite ACTs (C-ACT), kaolin ACTs (K-ACT), HRTs (containing activator and 6 units heparin) and PRTs (containing activator and either 0.04 or 0.08 mg protamine) were analyzed in either a Hemochron 801 or Hemochron 8000 dual chambered timer<sup>d</sup>. All test tubes used in this study contained 12 mg activator (celite or kaolin). For a given blood sample to be tested, 2 ml was added to the appropriate tube, the timer started,

the tube shaken vigorously until the activator was completely dispersed throughout the blood sample (approximately 3-5 sec) and then the tube was placed in the test well.

#### Heparin and Protamine Dose Quantification using the RxDx System

Heparin and protamine doses were calculated by linear regression of clotting time versus drug concentration. For heparin, the clotting time of the patient's blood sample at baseline (0 heparin) and in the HRT tube (3 USP heparin units/ml blood) were used to predict the heparin concentration in units per milliliter of blood, required to obtain the desired target time (480 seconds). For protamine, the clotting times for the status ACT (heparinized sample, zero mg protamine) and the PRT tube (0.02 or 0.04 mg protamine/ml blood) were used to calculate the concentration of protamine required in milligrams per milliliter blood to neutralize the heparin present. The calculated doses of heparin or protamine were then multiplied by the patient's estimated blood volume (6) to determine final dosing requirements (Figures 1A and 1B).

#### Effect of Aprotinin on Celite and Kaolin Based Clotting Times

Fresh blood samples were obtained from normal volunteer donors not receiving any prior medications. The number of donors for each analysis varied from two to twenty. Various concentrations of aprotinin<sup>b</sup> and/or beef lung heparin<sup>c</sup> were added to these samples which were then thoroughly mixed prior to addition of a 2 ml sample to ACT, HRT or PRT test tubes. Aprotinin concentrations used were selected to represent the complete range of plasma concentrations expected in a patient receiving the standard Hammersmith dose. Aprotinin effects were evaluated at 100 KIU/ml increments from 0 to 1000 KIU/ml. The average steady state intraoperative concentration of aprotinin in plasma from patients receiving a full Hammersmith dose ( $2 \times 10^6$  KIU bolus,  $2 \times 10^6$  KIU in the pump prime and  $0.5 \times 10^6$  KIU/hour continuous infusion) is 250 KIU/ml and 137 KIU/ml for patients receiving a half Hammersmith dose (3). Theoretical aprotinin concentrations, assuming complete distribution and little or no metabolism (500 and 300 KIU/ml) were also employed in the current study

### CLINICAL STUDIES

#### Patient Management

The clinical protocol was approved by the University of Pennsylvania Human Studies Committee. Consecutive patients (n=21) requiring primary elective cardiac surgery for coronary artery bypass grafting, mitral valve replacement, aortic valve replacement or any combination thereof were included. Patients who had previous cardiac surgery, a history of bleeding disorder or evidence of other organ dysfunction were excluded. All current cardiovascular medications were maintained until transport to operating room (including heparin) at which time

a HEMOCHRON<sup>®</sup>, International Technidyne Corp. (ITC), Edison, NJ

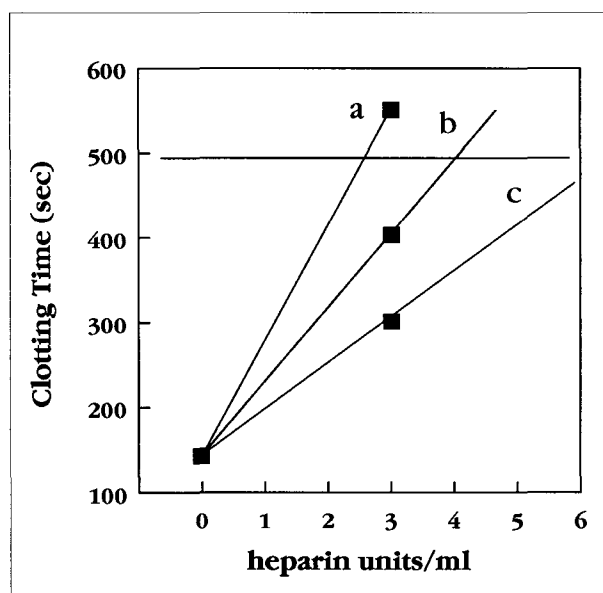
b Trasylol<sup>®</sup>, Miles, Inc., Pharmaceutical Division, West Haven, CT

c RxDx<sup>®</sup>, International Technidyne Corp., Edison, NJ

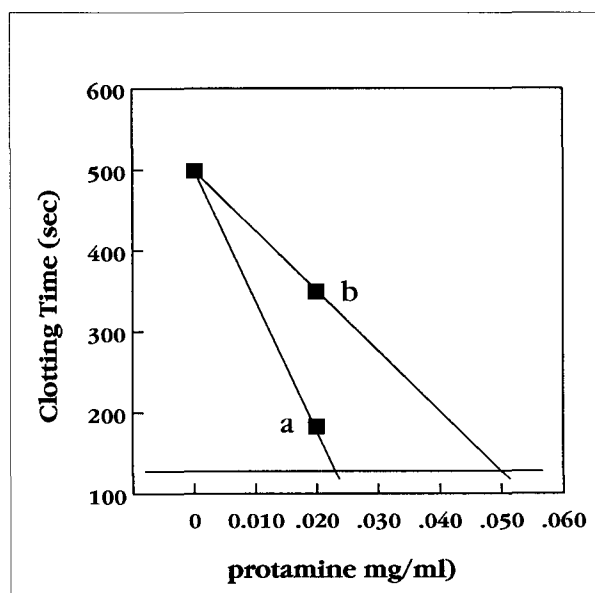
they were discontinued. All patients were premedicated with morphine sulfate and scopolamine, received a 16 gauge peripheral intravenous catheter, 20 gauge radial artery catheter, and pulmonary artery catheter via the right internal jugular vein before induction of anesthesia. A combination of high dose fentanyl, isoflurane or enflurane, and non-depolarizing muscle relaxant were used to produce anesthesia. Comparative heparin and protamine dose quantification using the celite and kaolin based RxDx systems were made for all patients. Celite and kaolin based tests were run simultaneously throughout each case. Baseline ACT and HRT tests were performed at several sampling times prior to systemic heparin administration at the time of arterial line placement, Swan-Ganz catheter placement, administration of anesthesia and following sternotomy. The heparin dose based on the celite RxDx result was administered

via the central venous line. Hypothermic bypass (26°C) was initiated when the C-ACT exceeded 400 seconds. ACT values were obtained five minutes following the heparin bolus dose and at 15 minute intervals during bypass. PRT values were obtained once patient rewarming had begun and the rectal temperature had reached 35°C. All patients received heparin (from porcine intestinal mucosa) and protamine which was matched by lot number to the preparations in the HRT and PRT test tubes. ACT, HRT and PRT tests were determined in duplicate using a Hemochron 801 dual chambered timer with a 300 sec prewarming feature. The appropriate test was placed in the timer test well for 300 sec to warm the glass tube and test well to 37°C. Ten ml of whole blood was aspirated from the arterial line before a sample for testing was drawn. ACT, HRT and PRT clotting times and predicted heparin and protamine doses

**Figure 1: Calculation of heparin and protamine dose using the RxDx system** Dosing calculations for *in vitro* studies were based on a hypothetical male patient, 178 cm tall and 81.6 kg with an estimated blood volume of 6.0 liters. Dose quantification is based upon linear regression analysis of clotting time techniques.

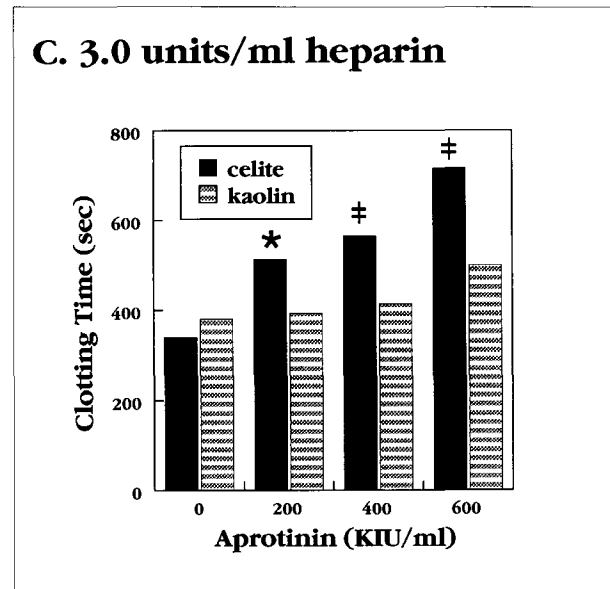
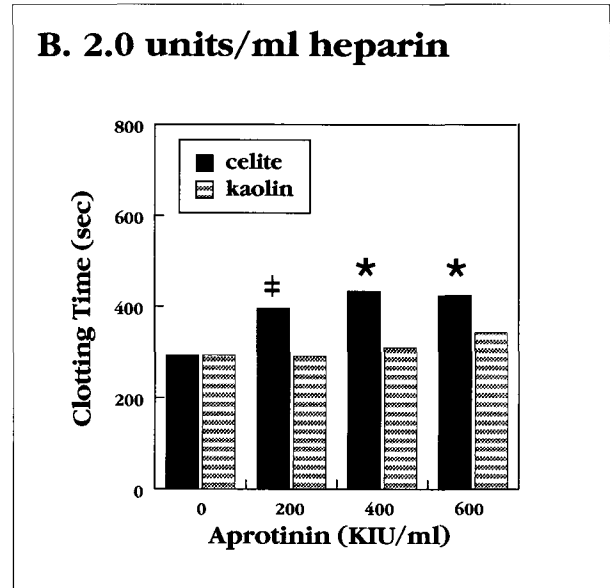
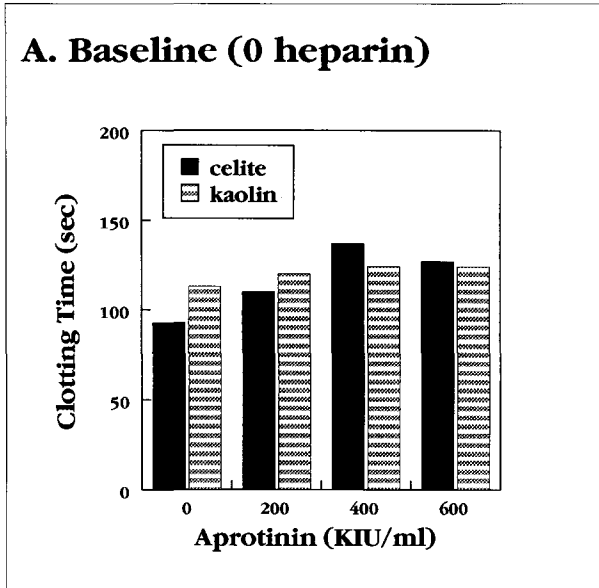


**A. Heparin Dose.** Calculation of required dose to reach a 480 second target time is presented for 3 hypothetical patients. The clotting time of a patient's blood is determined in an HRT tube which contains 3 USP heparin units/ml (3, on the x-axis). The more heparin resistant the patient, the shorter the HRT clotting time. Heparin dose is quantified based on the predicted units/ml required to reach the target ACT time multiplied by the patient's estimated blood volume. Patient 'a' is heparin sensitive and would require a dose of only 15000 units (2.5 units/ml x 6000 ml), equivalent to 180 units/kg, to reach target. Patient 'b' responds as expected to heparin, requiring a dose of 23000 units (3.8 units/ml x 6000 ml), equivalent to 280 u/kg. Patient 'c' is heparin resistant and would require a bolus dose of 36000 units (6.0 units/ml x 6000 ml), equivalent to 440 u/kg.



**B. Protamine Dose.** Calculation of required dose to reach a 120 second baseline is presented for 2 patients. The clotting time of the patient's blood is determined in a PRT tube which contains either 0.02 or 0.04 mg/ml protamine (0.02 and 0.04, on the x-axis). The choice of PRT tube is determined by the total amount of heparin (bolus + pump prime + subsequent doses) given throughout the case. Using either one of the PRT test tubes, the more circulating heparin present in the patient's blood, the longer the PRT clotting time. Protamine dose is quantified based on the predicted mg/ml required to return to the baseline ACT time multiplied by the patient's estimated blood volume. The two curves shown illustrate two patients, each receiving a moderate amount of heparin ( $\leq 43,000$  units). The total protamine dose for patient 'a' is 150 mg (0.025 mg/ml x 6000 ml). Patient 'b' requires a total protamine dose of 290 mg (0.048 mg/ml x 6000 ml).

**Figures 2A, 2B, and 2C: Sensitivity of Celite and Kaolin ACT tests to Aprotinin.** Results of an *in vitro* analysis of triplicate determinations using blood samples from 2 normal donors (N=6 for each bar). Statistically significant differences between Celite ACT and Kaolin ACT values are denoted as: \* ( $p < 0.05$ ) or † ( $p < 0.01$ ). The steady state plasma concentration of aprotinin after a full Hammersmith dose is 270 KIU/ml.



obtained using the celite and kaolin systems were compared.

**STATISTICAL ANALYSES**

All regressions, ANOVA and Student's t test analyses were performed using Statgraphics® Statistical Analysis software<sup>d</sup>.

**RESULTS**

**LABORATORY STUDIES**

**Effect of Aprotinin on Celite and Kaolin Based Clotting Times**

In the absence of heparin, aprotinin has no effect on baseline ACT values using either celite (clotting time range 93-134 sec.) or kaolin (clotting time range 113-137 sec.) based tests (Figure 2A,  $p > 0.2$ ). However, the baseline kaolin ACT is generally 5-10% higher than the comparable celite value ( $p=0.01$ ). In the presence of heparin, the celite based ACT is affected by aprotinin at concentrations as low as 200 KIU/ml, while the kaolin based ACT values are unaffected at aprotinin levels below 600 KIU/ml (Figures 2B, 2C). At levels above 600 KIU/ml, elevation of the kaolin based ACT is also observed (data not shown). The magnitude by which the celite ACT becomes artificially prolonged is proportional to the amount

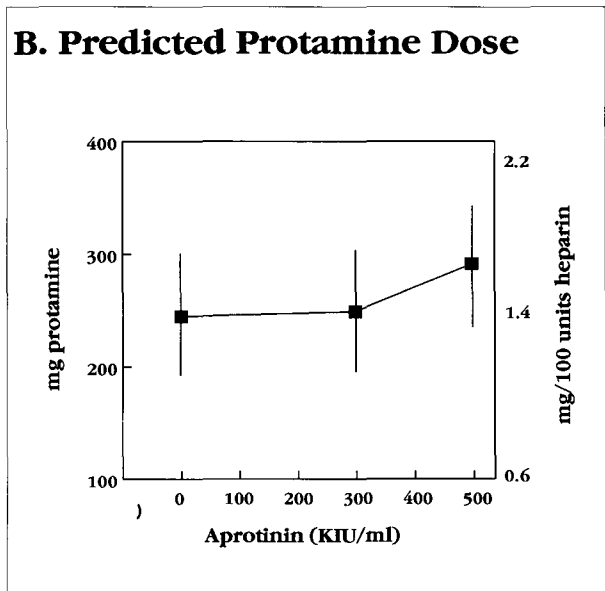
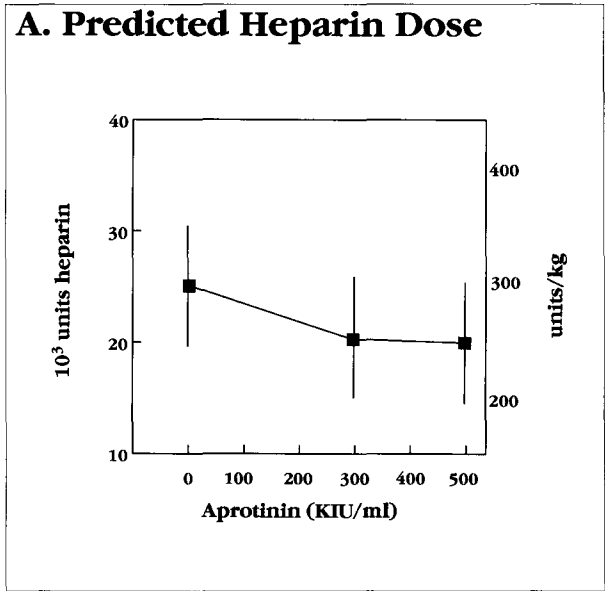
of heparin present (Figures 2B and 2C).

**Heparin and Protamine Dose Calculation Using Kaolin Based Assays**

For comparative purposes, heparin and protamine doses were calculated for representative patients using the HRT and PRT test results obtained from normal donor studies. In the presence of aprotinin, there is a small decrease in the predicted heparin dose from  $25600 \pm 6700$  units to  $20800 \pm 5200$  units

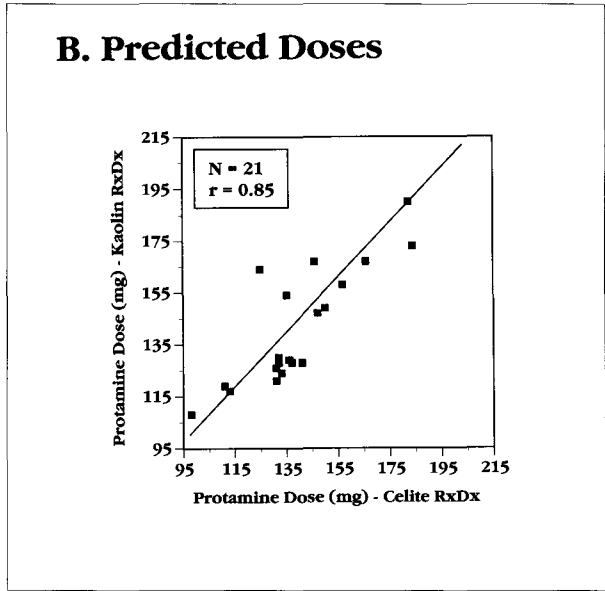
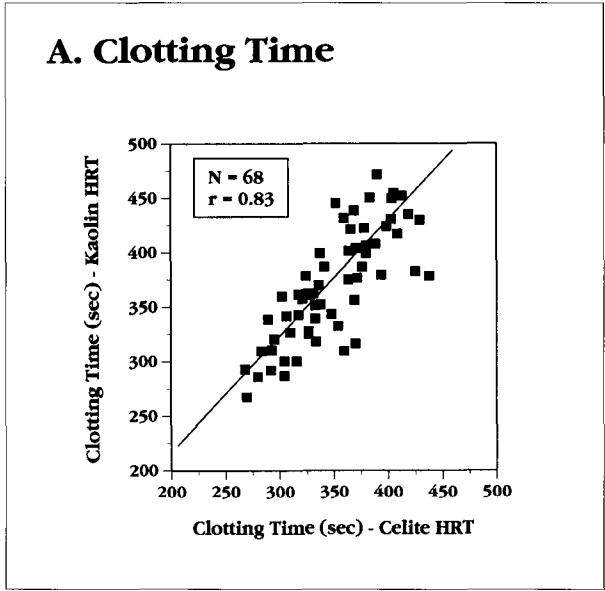
<sup>d</sup> Manugistics®, Rockville, MD

**Figures 3A and 3B: Comparative dose requirements using the kaolin HRT and PRT for samples containing aprotinin.** Effect of the addition of aprotinin to the quantified heparin and protamine dose. Analyses were performed using triplicate blood samples from 10 normal donors at aprotinin increments of 100 KIU/ml. Data for 0, 300, and 500 KIU/ml are presented.



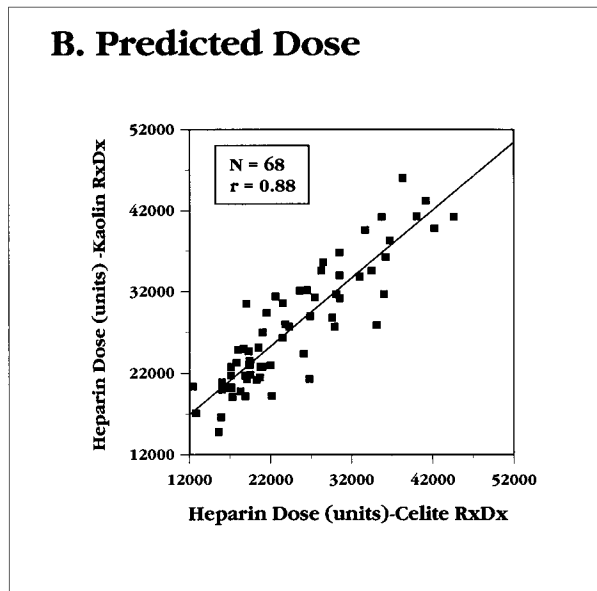
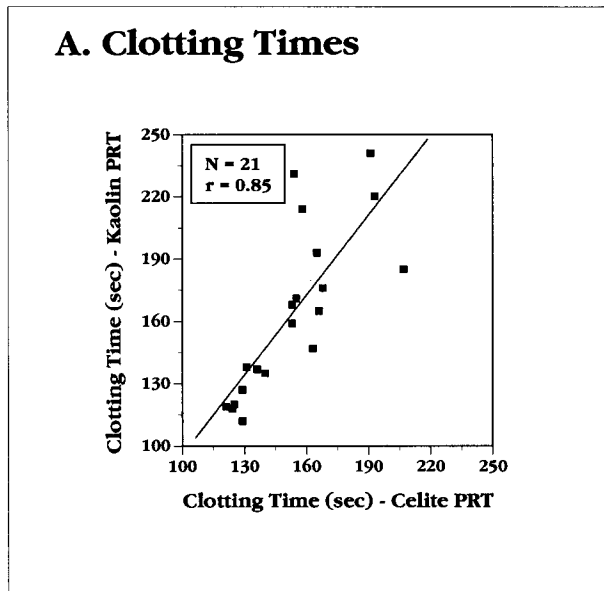
(Figure 3A). This decrease, while not significant, is evident at both 300 KIU/ml and 500 KIU/ml aprotinin levels (Figure 3A) but it is not observed at either 100 or 200 KIU/ml (data not shown). Aprotinin levels up to 300 KIU/ml have no effect on the predicted protamine dose (Figure 3B). At aprotinin levels of 500 KIU/ml, there is an increase in the predicted protamine dose from 248 ± 29 mg to 293 ± 82 mg. Neither the decreased

**Figures 4A and 4B: Comparison of predicted heparin dose using the celite and kaolin based HRT.** HRT tests were performed prior to systemic heparinization. Clotting times (A) were recorded and the heparin dose predicted (B) for each patient individually using patient specific baseline, HRT and blood volume values. Regression analyses were performed yielding the following: A.  $KHRT=1.08(CHRT) + 4.56$  where KHRT is the kaolin HRT clotting time and CHRT the celite HRT clotting time; and B.  $KRx Dx=0.87(CRx Dx) + 5358$  where KRxDx is the heparin dose calculated from the kaolin RxDx system and CRxDx is the heparin dose calculated from the celite RxDx system.



heparin dose nor the increased protamine dose observed at high aprotinin concentration were statistically significant ( $p > 0.20$ ).

**Figures 5A and 5B: Comparison of predicted protamine dose using the celite and kaolin based PRT.** PRT tests were performed during the patient rewarming period. Clotting times (A) were recorded and the protamine dose predicted (B) for each patient individually using patient specific baseline, PRT and blood volume values. Regression analyses were performed, yielding the following: A.  $KPRT=1.18 (CPRT) - 17.8$ , where KPRT is the kaolin PRT clotting time and CPRT is the celite PRT clotting time; and B.  $KRx Dx=1.07 (CRx Dx) - 4.05$ , where KRx Dx is the protamine dose calculated from the kaolin Rx Dx system and CRx Dx is the protamine dose calculated from the celite Rx Dx system.



**CLINICAL STUDIES**

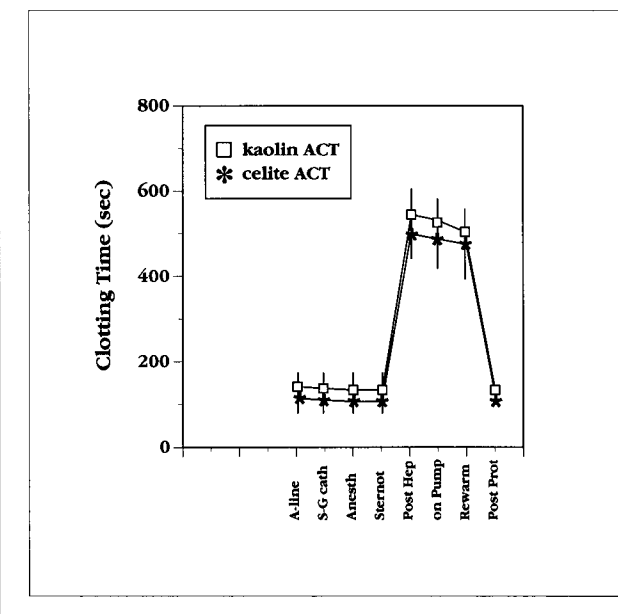
The celite and kaolin HRT clotting times (Figure 4A) and quantified heparin dose (Figure 4B) were highly correlated ( $r=0.83$  and  $r=0.88$ , respectively). The celite and kaolin PRT clotting times (Figure 5A) and the predicted drug doses (Figure 5B) were also highly correlated ( $r=0.85$  and  $r=0.84$ , respectively).

In these studies the HRT and PRT results are used to calculate the heparin and protamine dose based on each patient's blood volume. The anticoagulation profile of cardiac surgery patients over the course of the testing intervals is shown (Figure 6) as the mean  $\pm$  standard deviation for all ACT values obtained for the 21 patients. On average, at high heparin levels, the kaolin based ACT tests are approximately 5-10% longer than the celite based ACT performed concurrently ( $p < 0.01$ ).

**DISCUSSION**

These studies confirm the earlier observation (4) regarding artificial prolongation of celite based clotting time tests in the presence of aprotinin. In contrast to the dramatic effect on the celite assays, the effect on kaolin based tests is relatively minor at concentrations spanning the clinically relevant range. Employing a kaolin Rx Dx dosing system, heparin dosing differences were only observed *in vitro* when the aprotinin concentration was equal to or greater than 300 KIU/ml and prota-

**Figure 6: Anticoagulation profile of cardiac surgery patients: Comparison of celite and kaolin based monitoring.** ACT values (mean  $\pm$  standard deviation) are presented for both celite and kaolin ACTs at the time of arterial line placement (A-line), Swan-Ganz catheter placement (S-G cath), post-anesthesia induction (Anesth), sternotomy (Sternot), five minutes after heparin bolus administration (Post Hep), during cardiopulmonary bypass (on Pump), during the patient rewarming period (Rewarm), and after protamine administration (Post Prot).



mine dosing was affected at aprotinin concentrations equal to or greater than 500 KIU/ml. A concentration of 300 KIU/ml aprotinin is equivalent to a theoretical maximum plasma concentration after the administration of a half Hammersmith dose based on 100% distribution of the drug in the blood. However, as the reported steady state plasma level of aprotinin after a full Hammersmith dose is only 250 KIU/ml, the RxDx quantified heparin and protamine doses are clinically indistinguishable from those predicted in the absence of aprotinin.

In a preliminary clinical evaluation of the utility of the kaolin RxDx system in patients receiving aprotinin during cardiac surgery, a 77.4 kg female patient requiring reoperation for coronary artery bypass grafting was given a bolus dose of 30,000 units heparin (388 unit/kg) based upon a desired 450 second target time. The post bolus K-ACT value obtained was 452 seconds while the celite ACT value obtained simultaneously was above 1000 seconds. At the end of case, the predicted dose of 260 mg protamine (0.58 mg/100 unit heparin) resulted in a return of the K-ACT to baseline (110 seconds). In this case the *in vitro* findings of aprotinin insensitivity proved to be applicable to clinical patient management. Further studies are underway to confirm this observation.

Both the RxDx hemostasis management system and aprotinin treatment have been shown to significantly reduce both postoperative bleeding and transfusion requirements in patients undergoing cardiac surgery. The magnitude of this reduction has been reported to be similar for either approach (1,7,8). At this time, it is unknown whether the combination of heparin and protamine dosing with the RxDx system and aprotinin treatment would show a synergistic interaction thereby further reducing bleeding, transfusion requirements and improving patient outcome. Prior to this definition of a kaolin based system, any examination of the interaction of RxDx and aprotinin had been impossible due to the deleterious effect of aprotinin on celite ACT based tests. The availability of a reliable kaolin based RxDx enables clinicians to conduct the necessary prospective trials to evaluate this hypothesis.

The kaolin based RxDx system is universally applicable to all cardiac surgery patients, both those receiving aprotinin as well as those not receiving the drug. The 5 to 10% elevation of clotting times apparent in these studies using a kaolin ACT compared to a celite ACT suggests that the appropriate K-ACT target time for cardiopulmonary bypass procedures may be slightly higher than than traditional C-ACT target times. This requires further clinical evaluation. The kaolin based heparin and protamine management system has the potential to further improve the reduction of postoperative bleeding and transfusion requirements in these patients when used in combination with agents such as aprotinin.

## REFERENCES

1. Jobs, DR, Aitken, GL, Shaffer, GW. Increased accuracy and precision of heparin and protamine dosing reduces

blood loss and transfusion in primary cardiac surgical patients. *J Thorac Cardiovasc Surg.* 1995;110:36-45.

2. DeLaria, GA, Tyner, JJ, Hayes, CL, Armstrong, BW. Heparin-protamine variability, a controllable factor in bleeding in open heart surgery. *Arch Surg.* 1994;129:945-950.
3. Miles, Inc., Pharmaceutical Division. West Haven, CT. Traysylol (aprotinin injection) package insert.
4. Wang, JS, Lin, CY, Hung, WT, Thisted, RA, Karp, RB. In vitro effects of aprotinin on activated clotting time measured with different activators. *J Thorac Cardiovasc Surg.* 1992;104:1135-40.
5. Wang, JS, Lin, CY, Hung, WT, Karp, RB. Monitoring of heparin-induced anticoagulation with kaolin-activated clotting time in cardiac surgical patients treated with aprotinin. *Anesthesiology.* 1992; 77: 1080-84.
6. Allen, TH, Peng, MT, Chen, KP, Huang, TF, Chang, C, Fang, HS. Prediction of blood volume and adiposity in man from body weight and cube of height. *Metabolism.* 1956;5: 328-45.
7. Murkin, JM, Lux, J, Shannon, NA, et al. Aprotinin significantly decreases bleeding and transfusion requirements in patients receiving aspirin and undergoing cardiac operations. *J Thorac Cardiovasc Surg.* 1994;107:554-6.
8. Lemmer, Jr, JH, Stanford, W, Bonney, SL, et al. Aprotinin for coronary bypass operations: Efficacy, safety, and influence on early saphenous vein graft patency. *J Thorac Cardiovasc Surg.* 1994;107:543-53.