The Heart Surgery Forum #2004-1134
7 (6), 2004 [Epub November 2004]
doi:10.1532/HSF98.20041134

Online address: www.hsforum.com/vol7/issue6/2004-1134.html

In Vivo and In Vitro Evaluation of the Heparin Management Test versus the Activated Coagulation Time for Monitoring Anticoagulation Level in Aprotinin-Treated Patients during Cardiac Surgery

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ABSTRACT

Introduction: Monitoring whole blood anticoagulation therapy with the activated coagulation time (kaolin ACT) and the heparin management test (HMT) were correlated in vivo with the plasma anti–activated factor X (anti-Xa) heparin concentration in patients who received variable doses of aprotinin and in vitro in the presence of increasing concentrations of aprotinin.

Methods: In 38 elective cardiac surgical patients who received an average heparin dose of 400 IU/kg and an average total aprotinin dose of 3.6×10^6 kallikrein-inhibiting units (KIU), ACT and HMT were measured in duplicate 6 times intraoperatively at predetermined intervals. Blood samples at each interval were also assayed for the anti-Xa plasma heparin concentration with the IL Test heparin chromogenic assay. The influence of increasing concentrations of aprotinin on HMT and ACT was also measured in vitro by using blood samples containing 6 IU/mL heparin from 6 additional patients after adding specific aliquots of aprotinin to achieve concentrations of 50, 100, 200, and 300 KIU/mL aprotinin. Linear regression analysis was used to compare HMT and ACT against anti-Xa. A P level <.05 was required for statistical significance.

Results: Duplicate measurements were taken at all intervals, and HMT and ACT values were significantly correlated, both with each other (r = 0.86; P < .01) and with anti-Xa activity (HMT, r = 0.81 [P < .01]; ACT, r = 0.71 [P < .01]). Aprotinin prolonged both the kaolin ACT and the HMT time in a dose-dependent manner (P < .05), and its influence was significantly less in vivo on the HMT time than on the kaolin ACT (P < .001).

Conclusions: The abilities of the HMT and the kaolin ACT to measure anticoagulation effects were not significantly different. Aprotinin prolonged both the kaolin ACT and the HMT time in a dose-dependent manner, but the HMT was significantly less affected by aprotinin in vivo. The HMT is a reliable alternative to measuring the ACT in cardiac operations and may offer greater accuracy in aprotinin-treated patients.

Received September 10, 2004; accepted September 13, 2004.

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INTRODUCTION

Precision in monitoring heparin therapy and its reversal is important in the successful conduct of cardiac surgery using cardiopulmonary bypass (CPB) or other major procedures using CPB. Both the timing and the high doses of heparin that are administered and reversed necessitate point-of-care testing to rapidly and accurately evaluate the adequacy of both anticoagulation treatment and hemostasis in the operation room.

The activated coagulation time (ACT) test is among the most frequently used tests of whole blood–based monitoring for determining the adequacy of anticoagulation treatment and its reversal in the operating room. However, previous studies have shown significant disparities in heparin dose, heparin effect, and heparin concentration [Culliford 1981, Esposito 1983, Gravlee 1988, Murray 1997]. Operator technique, patient hypothermia, hemodilution, the type of activator, and the administration of antiprotease drugs such as aprotinin can limit the accuracy of ACT testing [Cohen 1980, Moorehead 1984, Dietrich 1990, Wendel 1993, Despotis 1996]. The heparin management test (HMT) is a whole blood coagulation-monitoring system recently introduced in clinical practice as an alternative to current ACT measurements.

The purpose of this study was 2-fold: (1) to compare the diagnostic performance of the HMT with the conventional kaolin ACT test with respect to the measurement of heparin concentration in patients undergoing a variety of cardiac surgeries using CPB, and (2) to evaluate whether ACT and HMT measurements are affected by aprotinin in clinically relevant concentrations.

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METHODS

In Vivo Studies

After obtaining institutional review board approval and written informed consent, we investigated 38 patients who underwent elective cardiac surgical operations at the London Health Science Center, London, Ontario, Canada, between January and June 2003. Patients between the ages of 30 and 75 years who were to undergo elective coronary or valvular heart surgery using CPB were enrolled in the study. Exclusion criteria included emergency procedures and the administration of heparin within 12 hours preoperatively. All patients underwent normothermic nonpulsatile CPB using a membrane oxygenator and a 40-μm arterial-line filter primed with a total crystalloid volume averaging 1500 mL.

All patients were anesthetized by means of an opioid-based fast-track technique supplemented with inhaled anesthetics, muscle relaxants, and benzodiazepines. Heparin (Organon, Toronto, Ontario, Canada) was administered immediately before cannulation for CPB and as necessary to maintain the kaolin ACT greater than 480 seconds. Aprotinin was administered intraoperatively to selected patients on the basis of clinical considerations and in various doses, as a pump prime dose with or without a pre-CPB bolus and/or via continuous-infusion doses of 0, 2, 4, or 6 million kallikrein-inhibiting units (KIU). Protamine sulfate (Sabex, Boucherville, Quebec, Canada) was administered for heparin reversal after the patient was separated from CPB.

For this study, all HMT tests (Bayer, Tarrytown, NY, USA) and ACT tests (Actalyke; Array Medical, Ashford, Kent, UK) were performed from single blood samples that were divided and analyzed simultaneously and in duplicate at each interval. All samples were obtained from an indwelling radial artery catheter after the removal of 5 dead-space volumes to reduce the potential for heparin-flush contamination. During CPB, the samples were obtained directly from the venous reservoir of the membrane oxygenator. Data consistency was ensured by having 1 person process all of the samples. Both ACT and HMT tests were commenced immediately within 10 seconds of blood being drawn. ACT and HMT times were measured in duplicate at 6 times: (1) at baseline, (2) after heparin administration, (3) after 15 minutes and (4) after 30 minutes following the initiation of CPB, (5) before the end of CPB, and (6) after protamine administration. Four milliliters from each sample were used to evaluate the kaolin ACT in duplicate, and 100 μL (2 drops) of each sample were used to obtain HMT values in duplicate. An additional 2.7 mL of blood from the same sample was put into sodium citrate tubes (Vacutainer; BD Medical Systems, Franklin Lakes, NJ, USA) for subsequent determination of anti–activated factor X (anti-Xa) levels.

HMT and ACT values were compared by using an assay of anti-Xa activity to evaluate their diagnostic accuracy. The anti-Xa activity assay is performed in the coagulation laboratory to specifically measure plasma heparin levels by assessing the drug's interaction with Xa. This method uses diluted plasma that is mixed with antithrombin III and incubated with Xa. The residual Xa is mixed with a chromogen to reach a colorimetric end point. The heparin level is inversely proportional to the color developed. Because the anti-Xa assay is a plasma-based test and is performed in a standard laboratory, it is considered the gold standard for clinical monitoring of heparin concentration in the plasma [Teien 1976, Kovacs 1999]. ACT and HMT measurements were compared both with each other and with anti-Xa levels.

The ACT test was performed in duplicate by placing 2 mL of blood into prewarmed Actalyke kaolin ACT tubes, thoroughly mixing the tubes, and placing the tubes into each of the 2 rotating incubation wells of an Actalyke device. The basis of ACT measurement is mechanical mixing for the detection of fibrin clot formation. Each glass tube contains both kaolin and a magnetic rod that remains in a dependent position within the blood sample while the tube is in the measurement well of the device. With fibrin clot formation, the rod is engaged and rotated away from the sensor, which detects a change in magnetic attraction and automatically stops both tube rotation and the timer recording the time to clot in seconds.

HMT measurements were performed in duplicate with 2 separate Rapidpoint Coag machines (Bayer Health Care) and prewarmed cartridges. The HMT monitors clot formation via a proprietary microanalytic system using a flat capillary chamber on the surface of a credit card–sized disposable paper tablet. One drop (30-35 μL) of blood enters a reaction chamber containing paramagnetic iron oxide particles and freeze-dried reagents required for contact activation. For HMT measurement, the electromagnetically induced motion of the iron oxide particles in the blood sample is timed. When clotting occurs, the motion of the iron oxide particles ceases, and this change is detected by the analyzer, which records the clotting time in seconds.

The effect of aprotinin on ACT and HMT measurements in vivo was evaluated by dividing the total aprotinin dose administered to each patient by the body weight. Linear regression analysis was used to correlate HMT and ACT values drawn during CPB (intervals 3-5) with the weight-normalized aprotinin dose.

In Vitro Studies

For the assessment of the effect of aprotinin in vitro, 25-mL samples of whole blood were obtained from 6 additional patients before the induction of anesthesia and before the clinical addition of aprotinin or heparin. Samples were mixed with 150 IU (0.15 mL) heparin to achieve a final heparin concentration of 6 IU/mL, and aprotinin aliquots were then added in serial fashion to the heparinized blood samples to produce 5 different concentrations of aprotinin (0, 50, 100, 200, and 300 KIU/mL). At a constant heparin concentration of 6 IU/mL, HMT and ACT values were measured in duplicate following each serial addition over this range of aprotinin concentrations.

Statistical Analysis

Unless otherwise indicated, all data are presented as the mean ± SD. All statistical analyses were performed with SPSS software (version 11.5; SPSS, Inc, Chicago, IL, USA). The Pearson correlation was used to assess the relationship between ACT and HMT values and between these measures

Table 1. Clinical Characteristics of Patients in the In Vivo Studies*

Age, y	35-75
Sex (M:F), n	27:11
Cardiopulmonary bypass time, min	35-160
Duration of surgery, min	160-350
Total heparin, IU	30,000-95,000
Heparin, IU/kg	548 ± 127
Total protamine, mg	160-750
Protamine, mg/kg	3.45 ± 1.08
Aprotinin dose, $\times 10^6$ KIU	3.63 ± 2.27
Aprotinin/kg, $\times 10^4$ KIU/kg	4.78 ± 3.38

*Data are presented as the mean ± SD where appropriate. KIU indicates kallikrein-inhibiting units.

and anti-Xa activity. Analysis of covariance was used to assess the significance of the difference in slope between the correlations of ACT and anti-Xa activity and of HMT and anti-Xa activity for the in vivo and in vitro studies. The paired Student *t* test was used to assess the correspondence between the duplicate measurements with the 2 HMT monitors and between the 2 wells of the ACT machine. *P* values <.05 were required for statistical significance.

RESULTS

In Vivo Studies

Table 1 shows the demographic and clinical characteristics of the 38 patients. CPB duration averaged 94 ± 38 minutes (range, 35-160 minutes). An initial systemic anticoagulation level was achieved with heparin doses averaging 388 ± 58 IU/kg (range, 25,000-50,000 IU). The resulting heparin concentration at initial heparinization averaged 9.75 ± 2.40 U/mL (range, 6.16-15.2 U/mL). The total heparin dose was 45,000 ± 13,000 IU (range, 30,000-95,000 IU).

For the in vivo clinical study, 876 ACT and HMT measurements were performed on patient samples. Our analysis of the pooled data indicated that these measurements correlated well with each other ($r = 0.85$; $P < .01$), as is shown in Figure 1. The duplicate HMT values measured simultaneously with 2 separate Rapidpoint Coag monitors were not significantly different. For the baseline samples, the average ACT was 132 ± 15 seconds, and the average HMT time was 159 ± 44 seconds (Table 2). Five minutes after the first heparin bolus dose, the mean HMT time was 569 ± 103 seconds, and the ACT was 616 ± 144 seconds. During CPB, the average value, based on 2 or 3 samples from each patient, was 580 ± 79 seconds for the HMT and 635 ± 145 seconds for the ACT.

Table 2. Heparin Management Test (HMT) and Activated Coagulation Time (ACT) Measurements during Surgery*

Interval	ACT, s	HMT, s
(1) Baseline	132 ± 15	159 ± 44†
(2) After heparin	616 ± 144	569 ± 103†
(3, 4, 5) During CPB	635 ± 145	580 ± 79†
(6) After protamine	124 ± 13	160 ± 47

*Data are presented as the mean ± SD. CPB indicates cardiopulmonary bypass.

†$P < .01$, compared with corresponding ACT measurements.

HMT values at interval 2, 5 minutes after the first heparin dose, and at intervals 3 through 5 during CPB were significantly lower than the comparable ACT values ($P < .01$) (Table 2). HMT values at intervals 2 and 3 were not significantly different from each other ($P = .47$), but there was a strong trend for an increase in ACT over the same intervals ($P = .07$). An average protamine dose of 278 ± 100 mg was required for heparin reversal, and the HMT and ACT values returned to control values and were not significantly different from the baseline values (HMT, 160 ± 47 seconds [$P > .05$]; ACT, 124 ± 13 seconds [$P > .05$]). Consistent with interval 1 baseline measurements, HMT values at interval 6 after protamine administration were significantly higher than the comparable ACT results ($P < .01$).

As shown in Figures 2 and 3, both HMT and ACT results were closely correlated with anti-Xa levels ($r = 0.81$ and 0.71, respectively).

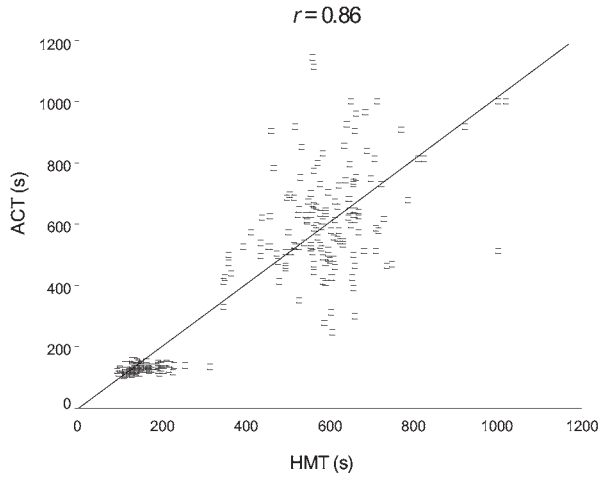

Figure 1. Relationship between the heparin management test (HMT) and the activated coagulation time (ACT).

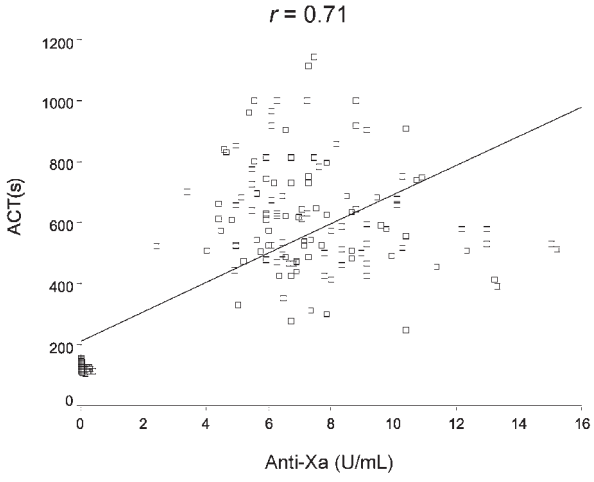

Figure 2. Correlation of the activated coagulation time (ACT) to the anti–activated factor X (anti-Xa) level in vivo.

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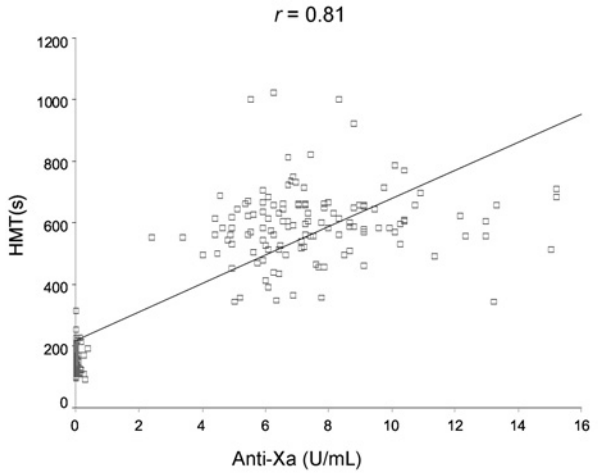

Figure 3. Correlation of the heparin management test (HMT) to the anti–activated factor X (anti-Xa) level in vivo.

Aprotinin Effect

Aprotinin added both in vivo and in vitro prolonged the kaolin ACT and HMT times in a dose-dependent manner ($P < .05$). For the in vitro studies, 30 parallel ACT and HMT measurements were performed in duplicate on blood samples from 6 patients. Aprotinin affected both ACT and HMT times in a dose-dependent manner ($P < .01$), as shown in Figures 4 and 5. The extent of aprotinin-mediated HMT time prolongation in vitro was less than for the ACT, but because of the small sample size, the difference was not statistically significant. In vivo, the aprotinin-mediated prolongation of HMT time was significantly less than the effect of aprotinin on kaolin ACT ($P < .001$) (Figures 6 and 7).

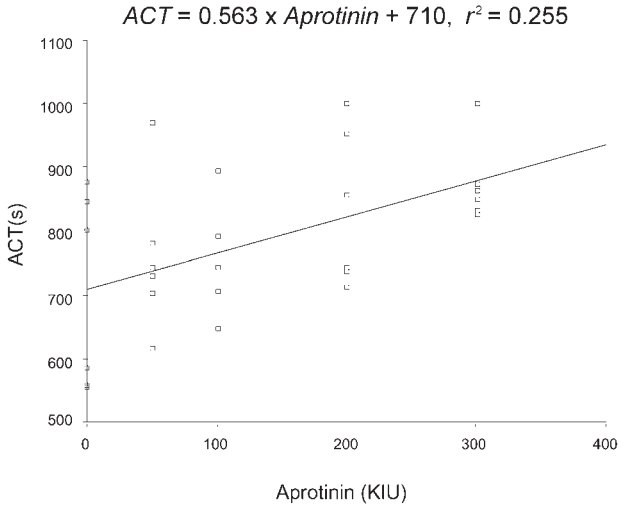

Figure 5. Linear regression analysis showing the correlation of activated coagulation time (ACT) to increasing aprotinin concentration in vitro in the presence of 6 IU heparin/mL. KIU indicates kallikrein-inhibiting units.

DISCUSSION

Precision monitoring of anticoagulation treatment and its reversal is very important in the management of patients undergoing cardiac surgery. The ACT is widely used to monitor anticoagulation treatment during cardiac surgery, but the accuracy of ACT is influenced by hemodilution, hypothermia, and aprotinin administration [Cohen 1980, Moorehead 1984, Dietrich 1990, Wendel 1993, Despotis 1996]. Conventionally, an ACT of 480 seconds or greater is considered adequate to initiate CPB, but the acute hemodilution that occurs

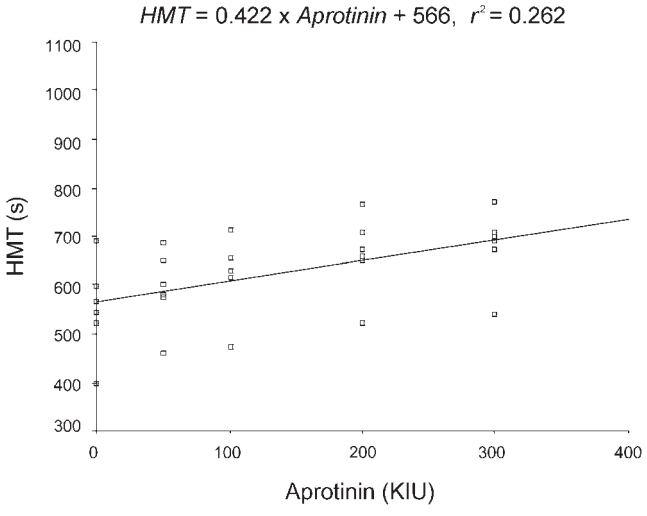

Figure 4. Linear regression analysis showing the correlation of the heparin management test (HMT) times to increasing aprotinin concentration in vitro in the presence of 6 IU heparin/mL. KIU indicates kallikrein-inhibiting units.

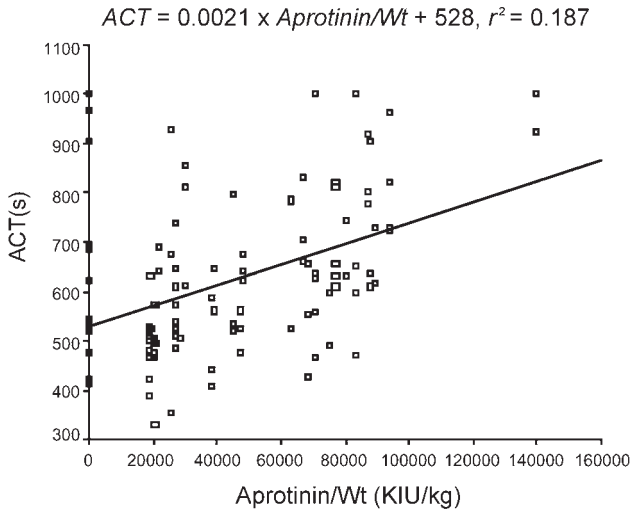

Figure 6. Linear regression analysis of activated coagulation time (ACT) versus total aprotinin dose expressed as kallikrein-inhibiting units (KIU) per kilogram body weight (Wt) and demonstrating a dose-related increase in ACT with increasing aprotinin concentration in vivo.

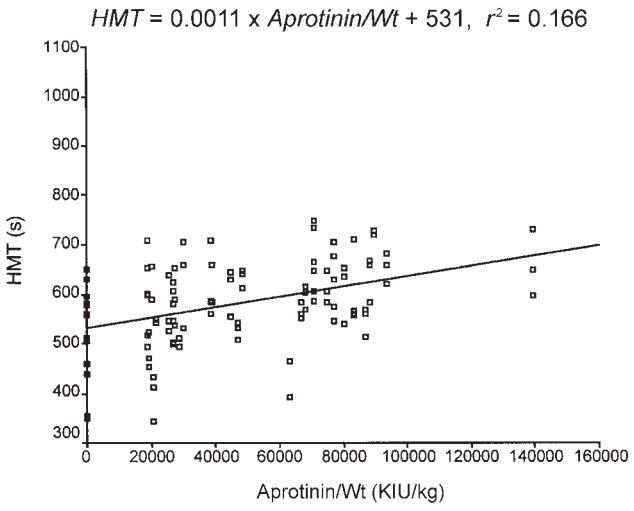

Figure 7. Linear regression analysis of the heparin management test (HMT) versus aprotinin dosage expressed as kallikrein-inhibiting units (KIU) per kilogram body weight (Wt) and demonstrating a dose-related increase in HMT time with increasing aprotinin concentration in vivo.

during CPB can produce an artefactual prolongation of ACT values. Although not statistically significant, the trend of an increase in ACT between intervals 2 and 3 demonstrated in the current study is consistent with this phenomenon.

The HMT performed by the Rapidpoint Coag analyzer is a point-of-care whole blood coagulation system recently introduced into clinical practice as an alternative method for the assessment of anticoagulation treatment [Bayer 1999]. The results of the current study show that the ACT and HMT values are well correlated, and both measures reflect the anticoagulation level as measured by the anti-Xa concentration.

Our study has shown that the HMT gives significantly and consistently higher values (averaging 13% higher than ACT values), both at baseline and after protamine administration. The postprotamine value in the HMT was not significantly different from the baseline value. However, the HMT values after the administration of heparin and during CPB were significantly lower than the comparable ACT values. This phenomenon of higher HMT baseline values and lower anticoagulated values than corresponding kaolin ACT values likely reflects differences in the HMT and ACT technologies used to assess clot formation.

Aprotinin prolonged the kaolin ACT and HMT times in a dose-dependent manner, both in vivo and in vitro; in vivo, however, aprotinin prolonged HMT times significantly less than kaolin ACT times. These results are qualitatively similar to those reported elsewhere. Despotis et al reported that aprotinin significantly prolonged both the celite ACT and the kaolin ACT in vitro and demonstrated that the aprotinin-induced prolongation was progressively greater at increasing concentrations of heparin ranging from 0 to 0.6 IU/mL [Despotis 1996]. Likewise, aprotinin in the current study prolonged both kaolin ACT and HMT times in vivo and in vitro in a dose-dependent manner in a direction similar to the results of Despotis et al. The magnitude of the effect observed here was greater than that observed by Despotis et al, likely reflecting our use of a 10-fold greater, clinically relevant heparin concentration of 6 IU/mL.

Wang et al reported that the kaolin ACT is less affected by aprotinin than the celite ACT [Wang 1992], most likely because of the greater potency of kaolin to activate coagulation and/or because of aprotinin binding by kaolin [Dietrich 1995]. In the current study, the range of aprotinin doses was 0 to 139,600 KIU/kg in vivo and 0 to 300 KIU/mL in vitro. These data clearly demonstrate that the prolongation of HMT times in vivo by aprotinin was significantly less than the effect of aprotinin on prolonging the kaolin ACT. In addition, although the magnitude of the aprotinin-mediated prolongation of HMT time in vitro was not significantly different from that of ACT, it was lower. Because 876 measurements were made in the in vivo phase of the study and only 60 measurements were made in the in vitro phase, a larger in vitro sample size would also have demonstrated a significant difference in the magnitude of the aprotinin effect.

In conclusion, the abilities of the HMT and the ACT to measure the anticoagulation level were similar when they were correlated with anti-Xa activity. Both tests are affected by aprotinin in a dose-dependent manner in vivo and in vitro, but the prolongation of HMT times by aprotinin in vivo was significantly less than for the kaolin ACT.

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