

The In Vitro Effects of Aprotinin on Twelve Different ACT Tests

Karen Jones, BS, CCP;* Fadi Nasrallah, MD;† Edward Darling, BS, CCP;* Nicole Clay, BS;* Bruce Searles, BS, CCP*

Departments of *Cardiovascular Perfusion and †Anesthesia, SUNY Upstate Medical University, Syracuse, New York

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Abstract: Aprotinin is frequently used during CPB to reduce post-operative bleeding and attenuate the inflammatory response. The level of anticoagulation in these patients is monitored by using various activated clotting time (ACT) tests, which are generally accepted as being altered by the presence of aprotinin in blood. Therefore, we have investigated the effect of aprotinin on several ACT tests using whole blood from CPB patients. With IRB approval, blood samples were collected from patients undergoing CPB before and after full heparinization (300 u/kg). Each blood sample was divided into two aliquots, and aprotinin was added to one of them to yield a final calculated concentration of 300 KIU/mL. Both aliquots were used simultaneously to perform the 12 ACT tests. A paired Student's *t*-test was performed on the data. Overall, test results from 9 of 12

devices were significantly increased by aprotinin. Of these, four were increased only when the sample was heparinized, three were elevated by both heparinized and unheparinized blood, and two were elevated only when the sample was unheparinized. Each affected test responded uniquely to aprotinin, producing ACT test results ranging from 12 to 51% above nonaprotinized values. Several tests that were affected by aprotinin using heparinized blood samples were unaffected using unheparinized blood samples. These data emphasizes the unique manner in which individual ACT tests respond to aprotinized blood samples and should be considered when developing institutional policy for anticoagulation of aprotinized patients. **Keywords:** activated clotting time (ACT), aprotinin, cardiopulmonary bypass (CPB), anticoagulation. *JECT. 2004;36:51-57*

In the United States, approximately 700,000 patients undergo cardiopulmonary bypass for heart and/or aortic surgery every year (American Heart Association, 1998 Statistics). Standard practice for these procedures includes heparin-induced anticoagulation that is monitored throughout the case using an activated clotting time (ACT) test (1,2).

Aprotinin is frequently used during cardiopulmonary bypass (CPB) for its anti-inflammatory (3,4), and antifibrinolytic properties (5,6,7), and for decreasing blood loss in post-operative CPB patients (5,8,9,10). It inhibits serine proteases plasmin and kallikrein, which mediate fibrinolysis, and also prevents heparin-induced platelet dysfunction (2).

When aprotinin is administered intravenously, it exhibits a dose-related plasma pharmacokinetic effect. It has been reported that a concentration of 200-250 KIU/mL is needed to inhibit kallikrein and a concentration of 50-125 KIU/mL to inhibit plasmin. Various dosing regimens

for aprotinin have been suggested, and a wide range of plasma aprotinin concentrations have been used clinically. One particular treatment is a high-dose regimen that requires two million KIU of aprotinin to be infused intravenously after the induction of anesthesia followed by an infusion of five hundred thousand KIU during the operation. Two million KIU of aprotinin is added to the prime of the CPB circuit (11). This high-dose regimen was used in a study by Dietrich et al., and an aprotinin plasma concentration of 335 ± 106 KIU/mL was measured 5 minutes after the onset of bypass in 20 primary CPB patients (5).

Numerous authors have suggested that ACTs are elevated in patients treated with aprotinin (7,12,13). Still, others have reported no elevation in the ACT in aprotinized patients (14). Although the exact mechanism of aprotinin's effect on the ACT is uncertain, it is clear that different ACT tests and activators respond differently under similar condition (15). Also, the literature describes only a few of the many ACT tests used clinically. Therefore, the purpose of this study was to investigate the effect of a clinically relevant aprotinin concentration on the results of many commonly used ACT tests using blood taken from patients undergoing cardiopulmonary bypass.

Address Correspondence to: Bruce Searles, BS, CCP, Department of Cardiovascular Perfusion, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY13210. E-mail: searlesb@upstate.edu

MATERIALS AND METHODS

Patient Selection

All patients scheduled for cardiac surgical procedures requiring extracorporeal circulation were considered for this Institutional Review Board (IRB)-approved study. Inclusion criteria required that patients be at least 18 years of age, have a hematocrit of 35% or greater, and a body surface area of 1.2 m² or greater. Jehovah's Witnesses, pregnant women, and patients with known coagulation disorders were excluded from the study. Written informed consent was obtained from each of the patients meeting the above criteria.

Activated Clotting Time (ACT) Tests and Machines

Twelve different point of care ACT tests and the specific machines used to perform each test are shown in Table 1. The performance of each machine was verified with electronic or liquid quality control tests according to manufacturer recommendations.

Conduct of Cardiopulmonary Bypass

Following anesthetic induction and after assuring hemodynamic stability, patients were systemically anticoagulated with 300 u/kg beef lung heparin. Cardiopulmonary bypass was initiated using a centrifugal pump, membrane oxygenator, and arterial line filter. Hypothermia to 30–34°C was achieved during bypass. The prebypass prime consisted of 1350 mL lactated Ringer's, 5000 units of heparin, 50 meq sodium bicarbonate, 12.5 g of mannitol, and 5 g of aminocaproic acid. Additional heparin was added during CPB to maintain an ACT greater than 400 seconds. The device used to measure ACT during CPB was the Sonoclot II Coagulation and Platelet Function Analyzer

(Sienco Inc., Morrison, CO). Heparin was reversed by protamine (1 mg/100 IU heparin) at the conclusion of the case.

Blood Sample Collection

Heparinized and nonheparinized blood samples were collected by a single technician, and each sample was divided into two aliquots (A1 and A2). Aprotinin was added, 0.03 mL of aprotinin per 1 mL of blood, to A2 to yield a final calculated aprotinin concentration of 300 KIU /mL. ACT tests were performed in duplicate using A1 and A2. Figure 1 illustrates the sampling and testing protocol. The sampling protocol was repeated at two time periods, before heparinization, and after heparinization while on CPB.

Comparison and Statistical Analysis

Duplicate tests were performed on A1 and A2. Test results from duplicate tests were averaged to define the experimental datapoint. When duplicate test results were not available, raw numbers were used. This sometimes occurred because of test malfunction. ACTs exceeding 1000 seconds were interrupted, and values less than 70 were eliminated from the data because of clinical irrelevance. Outliers were identified and eliminated according to Grubb's method for assessing outliers. The data for each test were expressed as an average \pm the standard deviation. Paired *t*-test was performed, and statistical significance was defined as a *p* value less than 0.05.

RESULTS

Table 2 lists, for each test, the comparison of ACT results with and without aprotinin, before and after full

Table 1. ACT tests and machines.

Test	Machines	Volume	Activator
MAX-ACT ^a	ActAlyke ^a	0.5 mL	Celite, Kaolin & Glass Particles
G-ACT ^a	ActAlyke	0.5 mL	Glass Particles
K-ACT ^a	ActAlyke	2 mL	Kaolin
C-ACT ^a	ActAlyke	2 mL	Celite
ACT ^b	GEM ^b	30–35 μ L	Kaolin
HR ACT ^c	HMS ^c	1 mL	Kaolin
ACT ⁺ ^d	Jr. Signature ^d	30–35 μ L	Kaolin
HMT ^e	Rapidpoint ^e	1 drop	Celite
FTCA 510 ^f	Response ^f	2 mL	Celite
FTK-ACT ^f	Response	2 mL	Kaolin
P214 ^f	Response	0.5 mL	Glass
SonACT ^g	Sonoclot ^g	0.36 mL	Celite

^aHelena ActAlyke (Helena Medical, Ashford, Kent, England).

^bGEM PCL (Instrumentation Laboratory Company, Lexington, MA).

^cHemochron Jr. Signature (International Technidyne Corporation Limited, Edison, NJ).

^dHemochron Response (International Technidyne Corporation Limited, Edison, NJ).

^eHepcon HMS (Medtronic HemoTEC, Englewood, CO).

^fRapidpoint Coag (Chiron Diagnostics Corporation, East Walpole, MA).

^gSonoclot II Surgical Analyzer (Sienco, Inc., Morrison, CO).

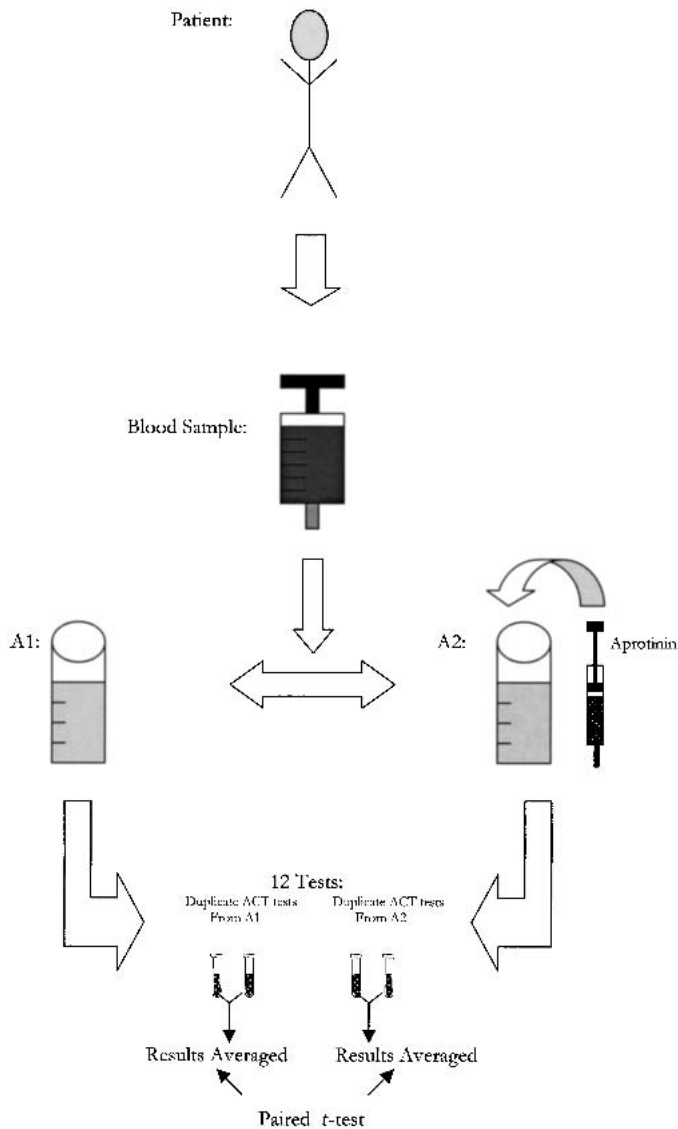


Figure 1. Illustration of the sample collection and processing protocol. A1: Aliquot 1 with no aprotinin; A2: aliquot 2 with aprotinin.

heparinization. Overall, 9 of the 12 tests were increased by aprotinin. Of these, three were increased with heparinized and unheparinized blood, and four were increased only when the aprotinized sample was heparinized.

Figure 2 illustrates the percentage change of ACT results with and without aprotinin for both heparinized and unheparinized blood samples. Four celite tests, five kaolin tests, one multiple activator test, and two glass bead tests were used.

For unheparinized blood samples (white bars), three of the celite tests were significantly elevated by the addition of aprotinin. The percentage change of these ACT tests ranged from 12–21%. For heparinized blood samples (black bars), four celite tests were significantly elevated by the addition of aprotinin. The percentage change of these ACT tests ranged from 27–51%. Three kaolin tests were significantly elevated by the addition of aprotinin to the heparinized blood sample. The percentage increase of the kaolin tests ranged from 4–16%. Both glass bead tests were significantly elevated using unheparinized blood samples (23–28% increase). These tests were not performed using fully heparinized blood because they are not designed for use with high-dose heparin blood samples.

Figure 3 shows the effect of aprotinin in unheparinized and heparinized blood samples on ACT test values. Of twelve tests, five were significantly increased with unheparinized blood samples. The change in ACT value in these tests ranged from 12–28%. Six of the 10 heparinized tests performed were significantly elevated. The change in ACT value ranged from 9–51%.

DISCUSSION

Aprotinin’s effect on ACT results has received a great deal of attention and has generated numerous scientific articles. There is a strong consensus that celite-activated test results are significantly elevated (16–24), and kaolin-

Table 2. Results of all tests performed.

Test	Activators	Machine	n	Unheparinized (s)		n	Heparinized (s)	
				A1	A2		A1	A2
Max-ACT	Glass, Celite, Kaolin	ACTAlyke	14	126 ± 13	129 ± 13	15	592 ± 252	603 ± 227
G-ACT	Glass	ACTAlyke	10	177 ± 21	229 ± 39*	N/A		
C-ACT	Celite	ACTAlyke	10	131 ± 15	161 ± 22*	10	513 ± 117	791 ± 156*
K-ACT	Kaolin	ACTAlyke	10	135 ± 11	138 ± 14	10	492 ± 53	588 ± 111*
ACT	Kaolin	Gem	13	120 ± 21	121 ± 20	11	462 ± 55	481 ± 60*
HR-ACT	Kaolin	HMS	13	145 ± 17	143 ± 13	15	571 ± 182	630 ± 201*
ACT+	Kaolin	Jr. Signature	14	132 ± 12	135 ± 11	13	496 ± 112	512 ± 53
HMT	Celite	Rapidpoint	14	161 ± 57	205 ± 52*	15	448 ± 73	618 ± 109*
FT/CA510	Celite	Response	13	144 ± 18	163 ± 17*	14	544 ± 133	903 ± 108*
P214	Glass	Response	7	132 ± 26	184 ± 15*	N/A		
FTK-ACT	Kaolin	Response	9	132 ± 19	139 ± 14	10	588 ± 80	569 ± 89
SonACT	Celite	Sonoclot	13	140 ± 39	147 ± 28	12	420 ± 63	852 ± 207*

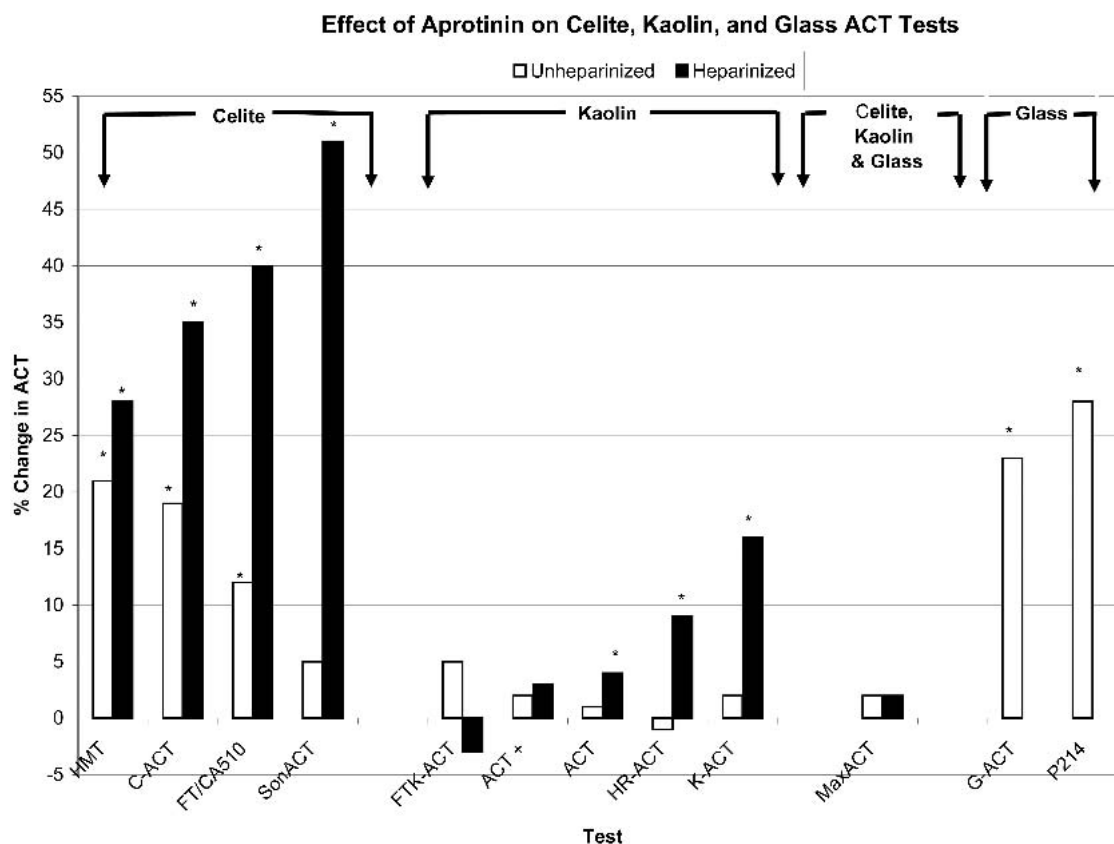


Figure 2. Percentage change of celite, kaolin, and glass activated clotting time tests. * Denotes significant difference ($p < .05$) between groups A2 and A1. This figure illustrates the percentage by which each test was changed by aprotinin.

activated test results are not significantly prolonged (14,17,19,20,23,24) by aprotinin. Our data do not refute the results of these authors but is in conflict with the current dogma, which suggests that all celite tests are affected by aprotinin, and all kaolin tests are not affected.

Historically, research on this topic has been completed using only a limited number of clinically available ACT tests. Of ten references regarding celite tests, only two different tests, ITC's FTCA510, (16,17,19–24) and Bayer's HMT (1,18), have been previously investigated. Furthermore, of the six articles referenced regarding kaolin tests, only two different tests were used. Five authors used or prepared a kaolin test similar to ITC's FTK-ACT, (14,19,20,23,24), while the other author's test was similar to Hepcon's HR-ACT (17,20).

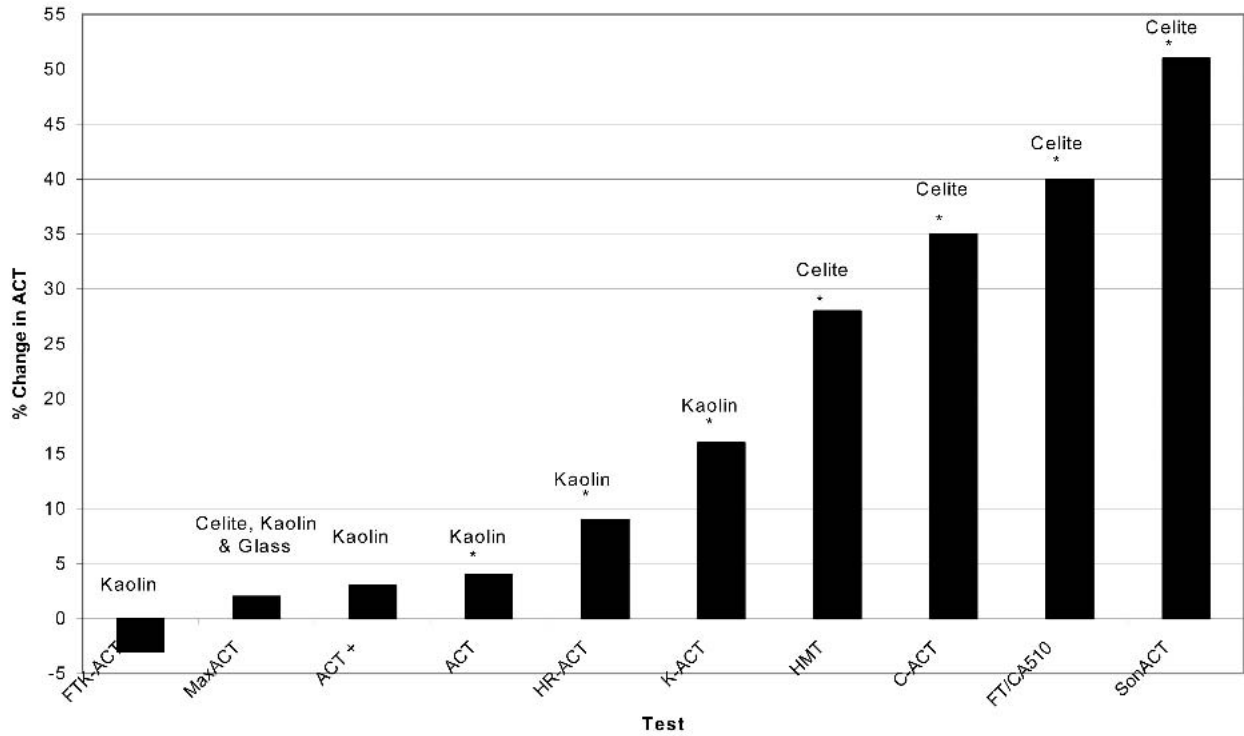
Many clinicians have extrapolated the results from these specific tests to different manufacturers' ACT tests with the same activator. This is inappropriate and has led to a misconception that is so persuasive that it has been printed in textbooks. The text *Cardiopulmonary Bypass: Principles and Techniques of Extracorporeal Circulation*, states that "this increase in ACT may be... only an in vitro effect that may be particularly pronounced with celite, but not

with kaolin as an activator." This demonstrates how the limited research has directed generalizations to all tests of the same activator.

Each of the tests investigated in our study responded to aprotinin uniquely, which contradicts the assumptions that all ACT tests of the same activator react to aprotinin in the same way. Regarding the celite-activated tests, our data support previous authors' findings of the FTCA510 test being elevated in the presence of aprotinin. This test was, in fact, elevated by aprotinin. All of the other celite-activated tests were also elevated but to different degrees. All of the celite tests had variable percentage changes in ACT.

Of the three kaolin tests investigated in our study, two of the tests were significantly elevated by the addition of aprotinin to heparinized blood samples. Their response, however, was less pronounced than the celite tests. The HR-ACT and K-ACT were both elevated by 9 and 16%, respectively, over nonaprotinized, heparinized blood samples. Our data support previous authors' findings regarding the FTK-ACT being unaffected by aprotinin. Literature on the HR-ACT kaolin test is inconclusive. Some authors have stated that with hepariniza-

Effect of Aprotinin on Heparinized ACT Tests



Effect of Aprotinin on Unheparinized ACT Tests

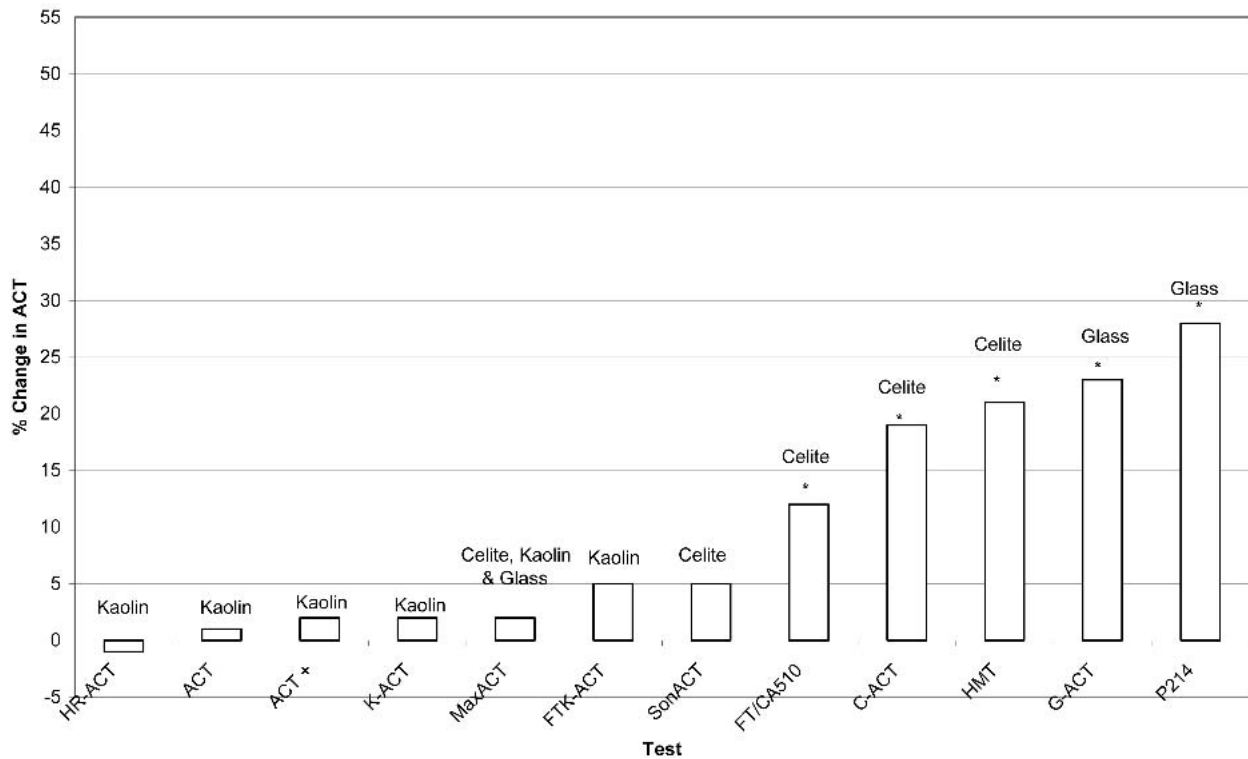


Figure 3. **A:** Shows the percentage changes of ACT values caused by aprotinin on all of the unheparinized ACT test, **B:** Shows the percentage change of ACT values caused by aprotinin on all heparinized ACT test. * Denotes significant difference ($p < .05$) between groups A2 and A1.

tion, the ACT value was elevated (16,25); whereas, others suggested there was no change in ACT with the HR-ACT test (17). Although no peer-reviewed literature is available regarding the Gem and Hemochron Jr. kaolin-activated ACT tests, we found they were not affected, which coincides with the product literature for these tests.

At the time of this study, no scientific or product literature exists regarding the effect of aprotinin on the K-ACT, G-ACT, C-ACT, MaxACT, SonACT, and P214 ACT tests. Our results demonstrate that all tests except the MaxACT resulted in increased ACTs in the presence of aprotinin. None was elevated to the same degree, illustrating the variability of all the tests.

The results of this study cannot be explained by the accepted margin of variability between duplicate tests. The variability of test results between the aprotinin and nonaprotinin samples shown in this study are greater than our group's previously reported variability between duplicate tests performed under similar conditions with only one exception (15). The variability of the Gem ACT variability between the aprotinin and nonaprotinin groups with heparinized blood was less than the reported variability of this test. This significant elevation in ACT between the aprotinin and nonaprotinin groups with the Gem ACT Test may not be attributable to the aprotinin but attributable to the normal variability of this tests result.

The variability between the tests investigated in this study may be influenced by the ratio of the volume of activator to the volume of blood. Dietrich et al. suggests that because aprotinin is positively charged, and kaolin is negatively charged the two molecules bind, thereby eliminating aprotinin's anticoagulation effect. Celite, being less negatively charged than kaolin, binds less aprotinin, leaving more to express its anticoagulation effect (26). If there is a high ratio of aprotinin to activator less would be bound leaving some aprotinin in the sample to apply its effects. If there is a low ratio of aprotinin to activator, then there will be enough activator to bind all of the aprotinin forbidding it to effect the anticoagulation of the sample.

Another theoretical explanation for the variability between tests may be proposed. Different manufacturers procure their activator agents from different vendors who ultimately may have different suppliers of the raw materials. Consequently, there may be qualitative differences between the celite or the kaolin used in the tests of different manufacturers.

In summary, ACT tests respond to aprotinin uniquely. Different manufacturers' tests that contain the same activator may react significantly, slightly, or not at all to the presence of aprotinin. Each ACT test should be considered separately and not grouped according to the activator

to predict the effect that aprotinin will have on the test results. These results should be considered when developing institutional policy on anticoagulation monitoring of aprotinized patients.

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