Review Article

Monitoring Anticoagulation During Aprotinin Utilization

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ABSTRACT

The literature reviewed discussed the varying practices of anticoagulation measurement in those open heart patients receiving aprotinin. All references have reported an increase in celite ACTs (C-ACTs) in heparinized patients who were treated with aprotinin. Two authors attributed this effect to aprotinin’s ability to enhance heparin’s anticoagulation and therefore permit a decrease in the heparin dose. Other authorities proved that during aprotinin administration the C-ACTs were artificially prolonged and that the C-ACT should either be maintained at 750 seconds or greater, or not be used at all. An alternative is the kaolin ACT (K-ACT), which is not affected by aprotinin except at serum levels above 500 KIU/ml. An additional method of measurement is the high dose thrombin time (HITT), a test that is not affected by variables that alter the C and K-ACTs but is inaccurate at low heparin levels. There appears to be no ideal method to provide an accurate anticoagulation measurement when considering aprotinin’s effect on the hemostatic system. Based on these data, the anticoagulation protocol remains an institutional decision in determining which measurement method will render cardiopulmonary bypass safe and effective when aprotinin is used.

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PHARMACOKINETICS OF APROTININ

Since its recent FDA approval, aprotinin\(^a\) has been a widely published and controversial drug. The ability of aprotinin to decrease blood loss during open heart surgery is well established (1). Aprotinin has various effects that pertain specifically to the components of the intrinsic clotting cascade, fibrinolysis and, to a lesser extent, platelet preservation. However, a current issue still in debate is the management of anticoagulation during cardiac surgery when aprotinin is used.

Aprotinin is a serine protease inhibitor derived from bovine lung. It is a polypeptide composed of 58 amino acids with a molecular weight of 6512 daltons. Aprotinin is metabolized via the renal system and has a half life of less than two hours.

Aprotinin has several proposed mechanisms of action (1-4). Most recently, researchers agree that the ability of aprotinin to improve platelet function is accomplished by the protection of the adhesive capacity of the platelet (2). The normal platelet is able to adhere to a damaged endothelial cell or subendothelial layer, through an affinity to adherence achieved by the platelet sticking to collagen and basal membrane structures. The interaction is supported by a bridge of the multimeric form of the von Willenbrand factor from the endothelium/subendothelium to the platelet at the glycoprotein (GpIIb) receptor site. The platelet subsequently undergoes a shape change, and spreads over the injured microvascular surface with exposure of different glycoproteins, such as GpIIb/IIIa complex, which can bind to fibrinogen (1). Current evidence suggests that aprotinin administration is associated with the preservation of the GpIIb receptor site during extracorporeal circulation.

This action is extremely beneficial, considering the detrimental effects on platelets during cardiopulmonary bypass (CPB). GpIIb receptors decreased by 50% in untreated patients within the first 5 minutes of CPB, while they were unaltered in the aprotinin group (2). This study suggests that aprotinin improved hemostasis during and post CPB by preserving this receptor, which is specifically damaged by the passage of blood through the extracorporeal circuit (2).

The traditional enthusiasm for aprotinin lies in its ability to minimize peri- and postoperative blood loss due to its antifibrinolytic and procoagulant qualities, both of which are mediated by inhibition of serine proteases in the blood (1,2).

Aprotinin’s antifibrinolytic action is accomplished by the inhibition of the serum protease, plasmin. Fibrinolysis occurs when plasminogen binds to fibrin and is converted to plasmin, which dissolves fibrin strands (2). Aprotinin use, therefore, decreases this conversion and maintains the integrity of the clot’s fibrin network. Plasmin inhibition is achieved at an aprotinin plasma level of 50 KIU/ml (1,2).

The coagulant action of aprotinin originates from its capacity to inhibit protein C. Protein C is a major regulatory protein that deters thrombus formation in the endothelial cell lining. Activated protein C is a serine protease, which can constrain the rate of thrombus formation by its own inhibitory actions on factors V and VIII in the intrinsic coagulation cascade (1). Because protein C is the precursor of a serine protease in plasma, it is inactivated by therapeutic levels of aprotinin (2). Thus, aprotinin can accelerate thrombus formation via this mechanism.

An enzyme of primary importance is kallikrein. The key factors induced by the activation of the kallikrein system are the effects on the intrinsic coagulation cascade and the responses mediated by contact activation (3). During CPB, kallikrein is converted from its circulating precursor prekallikrein, as the result of the activation of factor XII by the artificial surfaces of the oxygenator and tubing and by the exposed subendothelial tissues of the surgically cut vessels (3). Kallikrein is consequently formed in large amounts during CPB. The effects of kallikrein system activation are multiple. Kallikrein causes activation of the intrinsic clotting cascade due to its ability to activate factor XII. Kallikrein, conversely, has fibrinolytic properties that, once activated, cause the formation of a fibrinolytic protein, which accounts for less than one half of plasma fibrinolytic activity (5). Kallikrein, in addition, plays a role in the release of various substances that are associated with the inflammatory response (i.e. polymorphonuclear cells, leukocytes, complement, etc.). The contact of blood with the foreign surfaces of the oxygenator stimulates a large number of inflammatory cascades that can act through humoral or cellular mechanisms, but ultimately are controlled by amplification cascades of proteolytic enzymes. The vaso majority of inflammatory cascades are serine proteases (1). Thus, during CPB, by virtue of its ability to inhibit kallikrein, aprotinin can inhibit activation of the intrinsic coagulation cascade, attenuate fibrinolytic activity and lessen the impact of the inflammatory response (1,6).

The plasma level required to inhibit the kallikrein system is 200 KIU/ml, which is significantly higher than that which is needed to inhibit plasmin (1,2). The manufacturer’s literature (4) recommends a specific dosage pattern that maintains the plasma serum level at 200 KIU/ml. This is the standard dosing practice for aprotinin in those open heart patients receiving the therapy.

Due to the multiple effects of aprotinin on the intrinsic coagulation cascade, platelet function and fibrinolytic activity, there arises the challenge of determining the actual anticoagulation status. It is of paramount importance to ensure that patients undergoing CPB are adequately anticoagulated and thus avoid the detrimental events associated with inadequate anticoagulation.

ANTICOAGULATION MONITORING WITH APROTININ

Since the evolution of CPB and the subsequent need for systemic heparinization, the requirement for a fast reliable anticoagulant test was evident. The activated clotting time (ACT),
specifically the celite ACT (C-ACT), is the traditional anticoagulation measurement in the presence of high heparin doses. An ACT is performed by mixing the patient’s blood in a tube containing a surface activator. The contact of the blood with a foreign surface stimulates the intrinsic coagulation cascade and results in clot formation. However, an ACT is altered by numerous extraneous factors: hypothermia, hemodilution, thrombocytopenia, platelet inhibition, the type of surface activator and the presence of a contact activation inhibitor (like aprotinin) (5). Since aprotinin directly alters the coagulation status and the capacity for measurement, many studies have been done to determine an accurate method of anticoagulant measurement.

Some authors (7,8) state that aprotinin does have its own anticoagulant properties that can decrease the need for heparin. Others (3,6,9) state aprotinin’s procoagulant effects require much higher C-ACTs and continue conventional heparin dosage. There is also data reporting that the traditional method of measuring ACTs with celite is inaccurate and that a more precise level of measurement can be obtained by the use of the kaolin ACT (K-ACT) (11,12) or a high dose thrombin time (16,17). Finally, the manufacturer of aprotinin recommends that the standard dose of heparin be employed for CPB and subsequent dosing be administered on a fixed dose regimen or based on a quantitative heparin measurement technique unaffected by aprotinin, such as HMS$^{b}$ or HIT$^{c}$ (4).

In a randomized double blind trial by deSmet (7), aprotinin was given to one half of a sample group of 70 patients undergoing CPB. All patients received an initial heparin dose of 300 IU/kg and were given a subsequent dose of 2500 IU if the C-ACT fell below 400 seconds. More heparin was given to the non-aprotinin group because of lower C-ACTs, a finding which was statistically significant.

The researchers concluded that during CPB, anticoagulation by heparin can be synergistically enhanced by aprotinin, and that the need for heparin can be limited by aprotinin infusion (7). They formulated these conclusions based on the prolonged C-ACT and activated partial thromboplastin time (aPTT), a decreased AT-III consumption (from the inhibition of the intrinsic clotting cascade) and the lowered thrombin formation as measured by the fibrinopeptide A concentration, in the aprotinin group.

Similar conclusions were drawn by Feindt et al (8). These authors challenged the theory of requiring higher C-ACT during aprotinin use, and also investigated changes in heparin consumption and thrombin generation during its use.

The first clinical study by Feindt et al was a prospective randomized double blind study, in which 10 patients received placebo, while a similar number received the manufacturer’s recommended high dose of aprotinin (Regimen A) (4). All patients were heparinized with an initial dose of 200 IU/kg, and additional heparin was given (100 IU/kg) if the ACT fell below 480 seconds. Blood samples were drawn at various times, and the following tests were performed: C-ACT, d-Dimers, thrombin-antithrombin III complexes (TAT), aprotinin plasma concentration and fibrinogen split products (FSP).

The results indicated that a higher heparin dose was given to the placebo group corresponding with a higher circulating heparin level. During CPB the aprotinin plasma concentration in the treated group was between 150-300 KIU/ml. The aprotinin treated group had significantly lower FSP, d-Dimer levels, and TAT generation during CPB. Conversely, the aprotinin group had significantly longer C-ACTs than the control group.

A second clinical study was done on a group of 10 patients undergoing coronary artery bypass grafting who received the standard dose of aprotinin. This group had two C-ACT samples drawn every five minutes during CPB. One ACT sample was run as is, while the other ACT sample was diluted, 1:1 with a 0.9% NaCl solution. Dilution was the measuring method in the experiment in order to eliminate the influential factor, aprotinin (8).

This study had identical initial ACT values of diluted and undiluted amoles under aprotinin therapy. Significant differences were obtained between the groups with heparin administration. The diluted samples had a continuous correlation of the ACTs with heparin concentrations, whereas the undiluted samples’ ACT values did not correlate with heparin concentration.

A similar ex-vivo trial was done using the dilutional principle in donor blood and measuring C-ACTs (8). One series investigated the influence of increasing heparin concentration on ACT in the diluted sample. The other series investigated the influence of increasing heparin concentration coupled with an increase in aprotinin concentration in the ACT.

The ex-vivo dilution studies showed no difference in the ACT values of the diluted samples with varying amounts of heparin. When aprotinin was added to the samples, significant differences were found, using heparin concentrations between 2-4 IU/ml, there were parallel shifts of the ACT/heparin concentration curves under aprotinin therapy in a defined concentration range of 200-300 KIU. The linearity of the slope showed a significant correlation of the ACT with the heparin concentration in these conditions (8).

Feindt and associates concluded that aprotinin significantly inhibits thrombin activation, consistent with the decreased levels of TAT complex generation, d-Dimer and FSP. They also concluded that the dilution of blood samples appears to be useful in ACT measurements, since a correlation between the ACT and heparin concentration in maintained despite the presence of aprotinin (8). Feindt summarized: “In our opinion, a simple increase of the ACT values during extracorporeal circulation with aprotinin to values above 750 seconds as demanded by Hunt et al is unnecessary. For one thing, on the basis of methodology alone, with such high values there is no longer linearity between the ACT and the heparin dose. For another, merely through an increase in the heparin dose, a missing correlation cannot be proven. It is also difficult to understand why the heparin dose
should be raised in the case of significant decreases in thrombin activation through aprotinin and lack of proof of increased clotting tendency” (8).

The findings from Hunt et al (3) do not support those made by deSmet and Feindt. The aim of this study was to provide guidelines for monitoring heparin levels via C-ACT in the presence of aprotinin during CPB.

In Hunt et al’s in-vitro study, blood samples with a heparin concentration of 3 IU/ml were mixed with varying doses of aprotinin. Anticoagulation was assessed through the C-ACT and aPTT. In a further study by the same group, blood was obtained from nine patients who underwent CPB for coronary artery bypass surgery. Blood samples were taken after 10 minutes of CPB and were then mixed with aprotinin to give concentrations in the range of 5-450 KIU/ml. The C-ACTs, aPTT and prothrombin time (PT) were then analyzed. The authors reported that aprotinin produced a dose dependent increase in ACTs in blood samples produced from patients on CPB and that as the dose of aprotinin increased, the prolongation of the ACT became more variable. Similarly, the drug also caused a dose dependent increase in the aPTT (with and without heparin), but there was no effect of aprotinin on the PT. In conclusion, this group of researchers stated that: “It has been suggested by deSmet et al that aprotinin could be used clinically as a heparin sparing agent during CPB in view of its effect on the ACT. We have shown as have others that aprotinin prolongs the ACT and aPTT indicating that it too, is an anticoagulant. However, aprotinin’s other effects on hemostasis are prothrombic in that it has antifibrinolytic properties that are not measured in the ACT, thus reducing heparin levels with the addition of aprotinin may potentiate thrombic problems” (3). In view of these findings, the authors advocate maintaining C-ACT levels above 750 seconds in those patients receiving aprotinin.

The findings of two other researchers, Taylor and Najman, are in accordance with those of Hunt (3). Taylor reiterates, levels of at least 750 to 800 seconds or more should be achieved during CPB to avoid the occurrence of diffuse intravascular coagulation related to inappropriately low ACT levels (6).

In addition, Najman further concludes that the aPTT was elevated in a dose dependent manner due to aprotinin’s inhibitory effect on the kallikrein system, and, since there were no changes in the PT, thrombin time (TT), and heptest, aprotinin’s anticoagulant effect observed by an increased ACT is not heparin like (9). Hence, the above authors are in agreement that although there was an increase in the ACT with aprotinin supplementation, it is not correct to assume that this could permit reduced heparinization.

Another approach to measuring anticoagulation during aprotinin therapy is the use of kaolin ACT (K-ACT). The prime advantage of using kaolin as the activator is that there appears to be less fluctuation of the ACT values in the presence of aprotinin (5). One author states: “The prolonged ACT values with the celite activator in response to heparin and aprotinin should not be tolerated in clinical application since they signify a total lack of correlation between anticoagulation and ACT. Thus, monitoring of heparin essential for CPB did not work using celite as the activator” (10). K-ACTs can be measured using the same automated timing device as the C-ACTs, and therefore possess the same attributes (rapid, convenient, easy to use). K-ACT tubes are distinguished from the celite by their activator and contain kaolin (hydrated aluminum silicate) instead of celite (diatomaceous earth). Kaolin’s action parallels that of celite in that it is an activator of the intrinsic coagulation cascade via factor XII. However, it would appear that kaolin is a better contact activator than celite and that this activator still functions effectively in the presence of aprotinin (10). This effect is due to the different surface structure of kaolin compared to celite (10).

In an in-vitro study by Wang (11), 21 patients who underwent CPB were studied in double blind fashion. Patients received 200-300 IU/kg of heparin, and ACT samples were drawn at various intervals during the procedure. Both C and K-ACTs were measured on each sample, with the randomized addition of a specific amount of aprotinin in the concentration range of 80-180 KIU/ml. The ACTs measured pre-heparin and post-protamine showed no significant difference between the K and C-ACTs. Similarly heparinized blood samples without aprotinin showed no significant difference between K and C-ACTs. After heparinization the K-ACTs measured at any concentration of aprotinin correlated to the C-ACTs without the drug. Conversely, aprotinin at the various concentrations, 80-180 KIU/ml, prolonged the C-ACTs by 47-71%, in a dose dependent manner. The researchers concluded that the prolonged ACTs of heparinized blood in the presence of aprotinin were due to the use of celite activated tubes for measurement of ACT, and not to any inhibitory action of aprotinin on the coagulation system (11). The conclusions of this group supported the views of other authorities concerning the issue of decreasing heparin dosage as potentially dangerous in patients treated with aprotinin.

Wang (12) confirmed his in vitro findings in a second clinical study of patients treated with aprotinin who underwent cardiac surgery. The findings support the hypothesis that the higher ACTs of blood during CPB in patients treated with aprotinin are due to the use of celite as the surface activator, rather than to aprotinin-enhanced anticoagulation of heparin. The ACTs measured with the kaolin activator are insensitive to the presence of aprotinin and remain comparable to the ACTs in the control group (12). Wang and associates advocated maintaining the usual heparin dose and the use of K-ACTs as a measurement of anticoagulation in patients treated with aprotinin.

In a recent paper by Wendel and associates (10), they concurred with Wang’s findings. The fundamentals of Wendel’s study involved in vitro measurement of the levels of factor XII (FXII), pre-kallikrein (PKK) and kallikrein activity (KK) in donor blood samples. An in vivo study was accomplished comparing K-ACT and C-ACT in 20 patients who underwent coronary artery bypass surgery and were all treated with aprotinin. FXII levels were more reduced with kaolin than celite and this
difference was more pronounced in the presence of aprotinin. In addition, KK activities were more elevated with kaolin than with celite and the difference was more marked in the presence of aprotinin.

In summary, it would appear that kaolin is a better contact activator for correct ACT measurement during CPB with aprotinin (10). However, a potential source of error in monitoring the ACT via kaolin is the plasma level of aprotinin. One manufacturer of a K-ACT device states in their package insert that the heparin dose response curve was not altered in the presence of aprotinin (180 KIU/ml). As the level of aprotinin increased to 500 KIU/ml, the K-ACT became less precise in heparinized blood samples (13). Hence, the clinician must be aware of the potential alteration that could occur in the ACTs, especially if aprotinin plasma levels are not known.

In light of all the variables involving ACT measurement, a relatively new coagulation monitoring device called the High Dose Thrombin Test (HITT) is now available. Like the traditional thrombin time (TT), the test evaluates coagulation by measuring the formation of thrombin, and thus monitors the final common pathway. However, this method of measurement differs from the standard TT in its ability to measure the formation of thrombin in the presence of high heparin levels. The standard TT is very sensitive to low concentrations of heparin and therefore is inaccurate for the high heparin doses required during CPB. The manufacturer’s literature claims that HITT can monitor heparin levels from 200-500 IU/kg with a high degree of sensitivity and that HITT is not affected by hypothermia, hemodilution or minor decreases in fibrinogen, or the presence of FDP (14).

The efficacy of heparin level measurement using the HITT during CPB has been documented. Wang (15) evaluated coagulation activity in 27 patients undergoing CPB by using the C-ACT, K-ACT, and HITT. Samples were drawn at various times and the coagulation parameters assessed as in their previous study, in addition to the corresponding heparin level. He calculated a correlation coefficient between the heparin level and HITT, K-ACT, and C-ACT of 0.701, 0.534, and 0.104, respectively. The results indicated that HITT values had less individual variation in response to heparin and had a better correlation with the given heparin dose when compared to the ACTs (15).

Similarly, in a separate study, Huyzen and associates (16) examined the influence of aprotinin on the C-ACT, K-ACT, and HITT in 20 patients undergoing CPB. Three hundred IU/kg of heparin was administered to all patients with a subsequent dose of 100 IU/kg every 60 minutes. As before, the C-ACTs were significantly elevated in the aprotinin group versus the control group, whereas the K-ACT was not significantly higher in the group receiving aprotinin. Protamine administration returned both the K-ACT and C-ACT to baseline in both groups. Baseline HITT values were unable to be assessed because clot formation was complete in the non-heparinized samples before the reading was started. The same situation occurred after protamine administration and a HITT was not obtained. After heparinization, the HITT was approximately 150 seconds and remained slightly lower after the initiation and duration of CPB in both groups. The determination was that the HITT was more sensitive to the heparin AT-III levels than the ACTs. In contrast to the ACTs, the HITT decreased after the institution of CPB. This indicates that the HITT is sensitive to varying fibrinogen concentrations, and therefore, HITT is primarily monitoring heparin AT-III concentrations (16).

Murkin reported similar results (17). He investigated the effects of aprotinin on HITT and C-ACTs in 11 patients who underwent coronary artery bypass surgery. Six patients received the standard aprotinin dose and the remainder received a placebo. The findings showed no significant difference regarding the HITT values between the groups at any time. The C-ACTs were elevated in the aprotinin group at all times and the heparin concentration, as determined via factors IIa and Xa assays, were not significantly different in the treated and nontreated groups. The authors theorized that in the presence of aprotinin, the C-ACT is falsely elevated relative to the heparin concentration, while the HITT remained uninfluenced, thus better reflecting the anticoagulation status (17).

However, there are faults inherent to the HITT system. The test is designed to measure clot formation in the presence of high heparin levels. Therefore a baseline and post-protamine measurement cannot be established as was seen in the Huyzen study (16). Also, there is insufficient information regarding a safe range of HITT values. The manufacturer’s literature (14) cites 35-51 seconds as the normal range for a sample of fresh whole blood, and estimates that an HITT range between 150-250 seconds is equivalent to a heparin concentration of 3 to 5 IU/ml, and an ACT of approximately 460-600 seconds.

Huyzen addressed this dilemma in his study and hypothesized that, by simultaneous measurement of HITT and ACTs, it appeared that in absence of aprotinin, ACTs over 400 seconds correspond with HITTs over 100 seconds (17).

A further disadvantage of the HITT system lies in the fact that this test involves a more complex procedure and has a greater potential margin for error, depending on the accuracy of the preparation procedure. HITT tubes are very temperature sensitive, and the measurement must be done at 37°C. HITT tubes must be prepared individually by mixing precise amounts of water and dissolved thrombin, which are then incubated. The major disadvantage of the HITT is that dissolved thrombin is not stable for long periods of time and must be freshly prepared (18). Thus the method does provide a reliable indication of the adequacy of heparinization, at the expense of convenience and precision of continuous movement.

CONCLUSION

In view of what has been stated, the task of assessing the anticoagulation status in the presence of aprotinin can be a problematic one. Firstly, the assumption of the anticoagulant properties of aprotinin, theoretically enabling the dosage of heparin to be decreased, is wrong. Current research, with the exception
of one paper (8), suggest that aprotinin’s anticoagulant activity is minimal, if any, and that the heparin dose should not be altered when aprotinin is administered. Secondly, the clinician must decide on the most appropriate method of assessing anticoagulation in the aprotinin-treated patient. Each method is not without drawbacks, as stated. The C-ACT is falsely elevated with aprotinin and the traditional 480 second threshold value appears to not necessarily reflect an adequate level of heparinization. These factors considered, some researchers recommend that if a C-ACT is to be used with aprotinin, an acceptable value is 750 seconds or over.

The K-ACT is being used as an alternative to the C-ACT because its values appear to be influenced less during aprotinin therapy. However, the K-ACT results have a limited level of accuracy and can’t be deemed reliable if the plasma level of aprotinin is in the range of 500 KIU/ml. The clinician should therefore be aware that if the recommended dosage/administration guidelines are not followed, elevated plasma levels and thus inaccurate results could occur. An additional limitation for both C and K-ACTs is that there is extrinsic coagulant activity and complement production that is not detected in either of these tests.

The limitations mentioned prompted the development of the HITT. The HITT, in theory, is a superior index for the assessment of anticoagulation. It is designed specifically for aprotinin use in the CPB environment and provides qualitative results rapidly. However, in practicality, the HITT is not the optimum testing method. This system cannot provide baseline measurements during periods of low dose or no heparin and requires meticulous attention to ensure proper test preparation and result accuracy. In addition, the cost and maintenance of this system is higher than that of the conventional ACT analysis method.

Obviously, there is no ideal method to measure anticoagulation status in the face of aprotinin. It is anticipated in the future that there will become available a better measuring method that does not possess the problems inherent to the systems reviewed here. Until then, each institution must examine the information critically and determine which method can best meet their patients’ needs to make anticoagulation monitoring during aprotinin administration safe and effective.

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REFERENCES

4. Package insert for Trasylol (aprotinin injection). West Haven, CT: Bayer Corp.