Use of Aprotinin Therapy in a Patient with Factor V Leiden

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Patients with an abnormality of coagulation or fibrinolysis may be more prone to thrombotic complications perioperatively. One of these prothrombotic states is the factor V Leiden abnormality, which produces a resistance to activated protein C (aPC). This defect is now recognized as the most common cause of increased thrombogenicity and is found in approximately 2%–5% of the world population (1–3). Aprotinin is a nonspecific serine protease inhibitor that reduces bleeding associated with many types of surgery (4,5), most often repeat open heart surgery (6). Activated protein C is a serine protease and is inhibited in citrated plasma by aprotinin at concentrations of 2.5–3 μM (500–600 kallikrein inactivation unit [KIU]/mL) or more (7,8). These data, from patients who are not resistant to activated protein C, have lead to the suggestion that this may be a mechanism that makes clot formation more likely with aprotinin therapy (9,10). If the laboratory-based data can be extrapolated to the clinical situation, then the administration of aprotinin to patients with the factor V Leiden defect should enhance the prothrombotic or hypercoagulable state. An opportunity to discover whether aprotinin would have this effect followed the presentation of a patient with the factor V Leiden abnormality for heart transplantation.

Case Report

The patient investigated was a 34-yr-old woman. There was no personal or family history of thrombotic episodes. She first presented with severe chest and upper abdominal pain. An electrocardiogram showed global ischemic changes, and a coronary angiogram showed occlusions in the left anterior descending and right coronary arteries. The patient required support with inotropic drugs and intraaortic balloon counterpulsation. A repeat coronary angiogram showed normal coronary arteries and architecture. At this stage, the possibility of a genetic defect leading to a hypercoagulable state was considered. These investigations, presented below, showed that the patient had the factor V Leiden abnormality.

After this acute thrombotic episode, the patient had severe dyspnea on minimal exertion and was referred to be considered for heart transplantation. Investigations showed a poorly contracting left ventricle with an ejection fraction of approximately 20%. In addition, the left ventricle contained a mural thrombus. Warfarin therapy was initiated in addition to her other anticardiac failure therapies. In late 1995, the patient underwent a successful orthotopic heart transplant. The donor heart action recovered rapidly, and the patient was weaned from bypass with no difficulties. Postoperatively, the drain loss was about 1600 mL, which is more than average and was attributed to her preoperative aspirin and warfarin therapies. Both of these therapies were given on the day of her surgery but not thereafter in this early postoperative period.

Her early perioperative course was otherwise uneventful until about the 10th day postoperatively when a pericardial effusion required formal drainage under general anesthesia. The patient made a rapid and uneventful recovery from this procedure.

Approximately 6 weeks after transplantation, the patient presented with swelling of the leg. She showed signs of thrombosis in the inferior vena cava. Angiography confirmed a 10-cm length of clot in the inferior vena cava extending to below the renal vein from the abdominal bifurcation. High-dose heparin therapy was started. Over the subsequent days, the patient developed upper abdominal discomfort, increasing tenderness over the liver, and progressive abnormalities of liver function tests. Repeat venography showed the thrombus was now lying above the diaphragm and was lodged near the anastomosis with the atrium of the orthotopic heart. An abnormality or anomaly in the original anastomosis was thought to be the reason for the thrombus being maintained in that position. A decision was made to refashion the anastomosis and perform a thrombectomy, procedures requiring repeat cardiopulmonary bypass.

Associated with this decision, a discussion took place concerning the possible risk or benefits of the use of aprotinin therapy to prevent bleeding associated with this reoperation procedure within 10 wks of the first operation. A number of studies of coagulation using thrombelastography, among other systems, were performed.

The patient was returned to the operating room, and after induction of anesthesia, the patient was given a test dose of aprotinin (1 mL or 10,000 KIU) followed by an initial dose of aprotinin of 2.86 mg/kg (40,000 KIU/kg). A similar quantity was added to the prime of the oxygenator. The right atrium was inadvertently entered during the reopening, and this

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Table 1. Results from Hypercoagulability Screen

Protein C activity	64%	(65–138)
Protein C antigen	79%	(69–134)
Protein S antigen total	121%	(72–120)
Protein S antigen free	76	(64–145)
Antithrombin III activity	94%	(96–132)
Antithrombin III antigen	81%	(72–117)
Lupus inhibitor	Negative by DRVT & KCT	
Resistance to activated protein C	2.25	(1.7–3.8)
Fibrinogen	5.2 g/L	(1.8–3.7)
Factor VII activity	63%	(67–206)
Factor VIII activity	292%	(58–182)
Dilute clot lysis time	22 h	(10–20)
PAI-1 activity (5 –17)	16.9 IU/mL	
t-PA activity	1.18 IU/mL	(0.93–1.73)
Prothrombin factor 1.2	3.02 ng/mL	(0.58–2.87)

Values in parentheses are the normal range values for the variable being measured.

PAI-1 = plasminogen activator inhibitor, t-PA = tissue-type plasminogen activator, DRVT = dilute Russell venom time, KCT = kaolin cephalin time.

necessitated establishment of cardiopulmonary bypass with some urgency. Heparin (300 IU/kg) was administered for anticoagulation. The celite activated clotting test (ACT) was >1000 s. At operation, a thrombus approximately 10 cm in length was removed from the inferior vena cava, and the atrial suture line was refashioned. Despite the need for urgent establishment of bypass, the patient lost only 240 mL of blood into the drain in the 12 h after this second procedure.

A heparin infusion was given for 5 days after the thrombectomy, during which time the patient's anticoagulation regime with warfarin therapy was reinstated.

The patient was receiving warfarin, which maintained an international normalized ratio of approximately 2 prior to her operations, and the results of the coagulation studies need to be interpreted in this light. The results of her hypercoagulability screen performed after recovery from the myocardial infarction are shown in Table 1. These show that, as anticipated, there is a relatively low concentration of the vitamin K-dependent factors.

Genomic DNA was isolated from whole blood and was used for the amplification, by a polymerase chain reaction, of the factor V diagnostic region known to be linked to the Leiden mutation. The method standard for this type of analysis was used (2) except that results of the amplification and subsequent enzyme digestion were analyzed on a 3% NuSieve (FMC, Rockland, WI) (agarose gel in 1× Tris-acetate-ethlyenediaminetetraacetic acid buffer containing 0.5 μg/mL ethidium bromide). *In vitro* enzyme-mediated amplification of the 267-base pair (bp) region from the factor V gene containing the mutation site is shown in Figure 1A. After the purification of the DNA fragment, this 267-bp fragment was subjected to restriction digestion using the enzyme Mnl 1. This enzyme is capable of differentiating between normal and mutation genotypes. Based on the fragmentation patterns produced, the presence or absence of the factor V Leiden mutation can be determined. The 200- and 67-bp DNA fragments were obtained after following Mnl 1 digestion of the previously amplified 267-bp DNA fragment, indicating the presence of the G→A transitional mutation

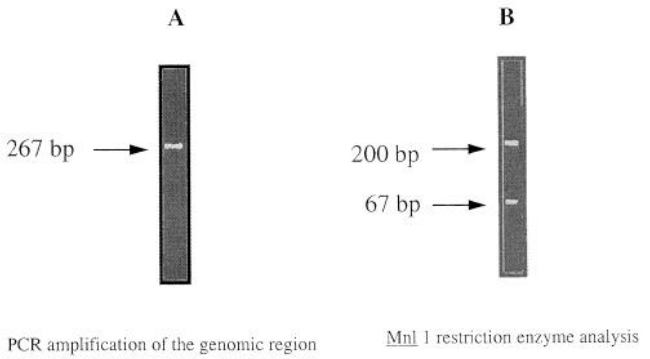

Figure 1. Scan of electrophoretic gel of DNA from patient with Leiden abnormality. The 267-basepair (bp) segment amplified by polymerase chain reaction is normally broken into fragments at 163, 87, and 37 bp by restriction enzyme Mnl 1. With the Leiden defect, only two fragments at 200 and 67 bp are shown.

within the gene (see Figure 1B). Blood from patients without the Leiden defect show bands at 163, 87, and 37 bp.

We wanted to determine whether the blood from this patient became more hypercoagulable with the addition of aprotinin. The thrombelastogram monitors the viscoelastic properties of the blood during clot formation. The time to clot formation is given by the r-time (normally 6–9 min). The rate of clot formation once initiated is given by the k-time (3–5 min) or α angle (>55°). The final clot strength is given by the maximum amplitude (MA) of the trace (40–60 mm). The thrombelastogram (TEG) thus provides a simple way of producing a functional assessment of an excessive rate or degree of clot formation (11,12). This is classified as r-time <4 min, k-time <1 min, or α >60° and/or a MA of >70 mm. Blood samples for testing were assayed within a minute of collection.

The TEGs were run in parallel with samples to which aprotinin had been added to achieve a number of final concentrations of aprotinin. Those samples with a final concentration of approximately 16 μM (800 KIU/mL) are presented here. A volume of saline equal to the aprotinin solution volume was added to patient control samples. The TEG was determined in three different sets of studies.

First, native and aprotinin-treated blood was allowed to clot under the normal conditions of thrombelastography. In these assays, the addition of aprotinin caused a marked prolongation of the r-time and a decrease in the α angle by approximately 30% compared with the control sample. The maximum amplitude was unaffected.

Second, to provide the supply of thrombomodulin necessary for the thrombin activation of protein C, these TEGs were repeated with the addition of cultured human microvascular endothelial cells. The final concentration of these cells was approximately 2000 per milliliter. The TEG with aprotinin and microvascular endothelial cells again showed prolongation of the r-time with no associated changes in either the α angle or the MA when compared with the control blood sample containing additional saline and cells. A representative TEG from these studies without and with aprotinin are shown in Figure 2 (A and B, respectively).

Finally, the TEG was developed in blood samples that had additional foreign surface activation with the diamataceous earth, celite, normally used to monitor the effects of heparin administration using the ACT. To perform this, activated TEG 2-mL samples of blood with or without aprotinin were added to Hemotech (International Techdyne, Edison, NJ)

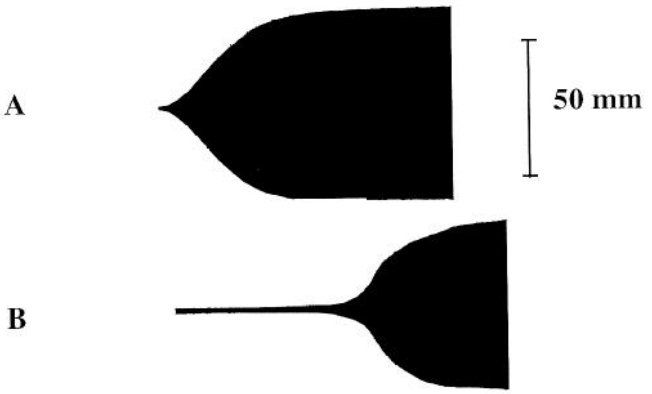

Figure 2. Scan of thrombelastography trace of blood from a patient with the Leiden deficiency without (A) and with (B) addition of aprotinin. Thrombelastogram A shows typical pattern of hypercoagulability with short r-time, increased α angle, and increased maximum amplitude.

CA510 ACT tubes. After gentle agitation, 0.35 mL of this blood was placed into the TEG cup, and the trace was allowed to develop in the normal manner. The ACT was also measured in these samples using a Hemotech 8000 (International Techdyne) coagulation monitor. Both the r-time and the ACT were prolonged (ACT from approximately 112 s to approximately 135 s) by the addition of aprotinin; the MA was unaltered.

Discussion

Blood coagulation is a complex series of interactions among formed elements in the blood, plasma factors, and the vascular endothelium. The mechanism of coagulation relies on an initiating stimulus, which progresses via an amplification process until there is the formation of insoluble fibrin strands. To prevent inappropriate or excessive activation, the body has a number of feedback mechanisms and control systems (Figure 3).

Factor V Leiden is produced by a single point mutation at position 1691 with a substitution of glycine for arginine, which eliminates the protein C cleavage site on activated or functional factor V (2). Because of this, the body's natural inhibition process at this point in the pathway is prevented (shown as the hatched area in Figure 3), and the process of coagulation can progress in an unhindered manner. Patients who have the factor V Leiden deficiet are known to have a greater instance of thrombosis, particularly in the venous system (13,14).

Aprotinin can inhibit a large number of the serine protease molecules found in the coagulation pathway (shown as the striped areas of Figure 3) at concentrations measured in humans receiving large-dose aprotinin (4). In the earlier parts of the cascade, other serine protease inhibitors in the plasma slow the generation of factor Xa. Aprotinin augments these actions and prolongs coagulation initiated by activation of both

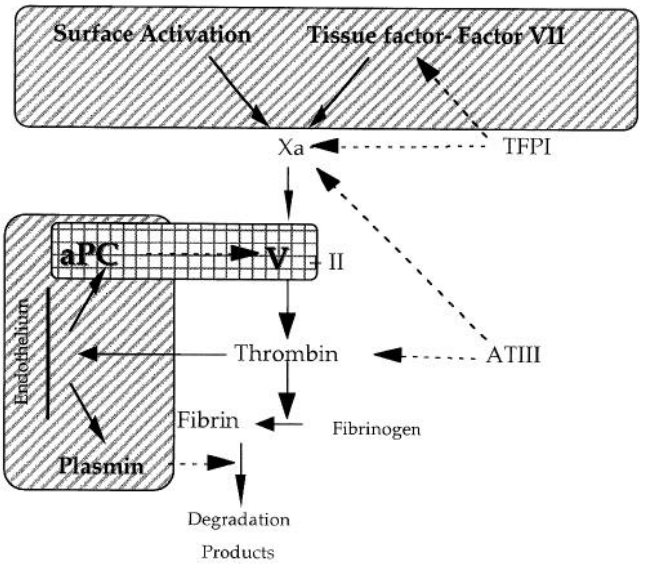

Figure 3. Schematic diagram showing coagulation factor pathways and inhibitors. Activation is shown by the solid arrow and inhibition by the dotted arrows. TFPI = tissue factor pathway inhibitor, AT III = anti-thrombin III, aPC = activated protein C. Light shaded areas are inhibited by aprotinin. Darker shaded areas show the defect in factor V Leiden that produces resistance to aPC. Potential overlap between inhibition of aPC by aprotinin and the genetic defect is clear.

intrinsic (15) and extrinsic (16,17) pathways. The increased r-time of the TEG with aprotinin in this patient and those reported by others (18,19) can be explained by these documented effects on these pathways. This effect also explains the reduced thrombin generation throughout cardiopulmonary bypass in the presence of large dose aprotinin therapy (4,20–25). Nonetheless, if thrombin is formed, then it acts both on fibrinogen to release fibrin and on the endothelium to produce substances that act as inhibitors of itself (aPC) or to increase fibrin degradation (t-PA) (Figure 2). aPC and t-PA are serine proteases. The role of the endothelium is therefore to increase the local concentration of serine proteases, which restrict fibrin generation.

Aprotinin will inhibit activated protein C. A concentration of approximately 500 KIU/mL produced a 50% inhibition of the enzyme, and at about 750 KIU/mL, there was a 90% inhibition (7,8). These studies and others investigating inhibitory actions were conducted in purified plasma systems. If aprotinin was able to functionally inhibit activated protein C in whole blood at these concentrations, then once coagulation was initiated, there would be a shortening of the k-time (or accentuation of the α angle) together with an increased MA, indicating a more hypercoagulable state due to the lack of negative feedback by aPC. Factor V Leiden deficiency should have amplified this process further. It was therefore somewhat surprising to find that, in the *ex vivo* studies, aprotinin did not seem to cause an overall increase in the likelihood or

rate of clot formation. In addition, our observations on the effects of aprotinin in the blood from the patient, but without endothelial cells, are similar to our previous observations and those reported for native and activated blood from presumed normal patients and volunteers (18,19).

These laboratory-based data were further supported by the fact that aprotinin did not generate an apparent hypercoagulable state in this patients' blood during the period of cardiopulmonary bypass. The celite-activated ACT was prolonged as anticipated with aprotinin therapy (26), and there was no evidence of clot formation during this second procedure. This argument would have been strengthened if we had performed assays of the TEG and other coagulation variables after administration of aprotinin to the patient and not simply to her blood. This had been our original intention. However, the need to administer heparin and establish bypass with some urgency prevented this.

It could be argued that if the patient was heterozygous for the Leiden deficiency, there was sufficient "normal" factor V to prevent protein C resistance. If this was the case, then it is difficult to explain her initial clinical presentation and the abnormal and significantly hypercoagulable baseline TEG shown in Figure 2A. In addition, there is little difference between the prothrombotic potential between the heterozygote and homozygote for this defect. There may be two possible explanations as to why aprotinin did not have an added deleterious effect on coagulation in the presence of the factor V Leiden abnormality:

1. Aprotinin may not inhibit activated protein C in whole blood at a concentration of 16 μM. This does, however, go against the findings of other studies in citrated plasmas (7,8).
2. In the case of resistance to aPC, other factors controlling coagulation may play an increased role in preventing a prothrombotic/hypercoagulable state from developing. If this was not the case, then patients homozygous for the defect should produce lethal thrombus early in life. These include tissue factor pathway inhibitor (TFPI) and antithrombin III. Other plasma protease inhibitors, such as α2 macroglobulin and α1 antichymotrypsin, or locally active protease inhibitors such as heparin cofactor II may also be involved. It is of interest and possible relevance that aprotinin has significant homology to TFPI (27). Aprotinin may be acting as a competitive agonist, similar to the action of TFPI, to produce an anticoagulant effect.

The likelihood of accelerated coagulation may contribute to an increased morbidity and mortality. We think that this case report is of interest both because of the unexpected hematological findings and because of its clinical significance. All the evidence so far points to patients with a hypercoagulable state becoming less hypercoagulable when given aprotinin and other serine protease inhibitors (18,20–22,25). The precise mechanism by which aprotinin acted to inhibit rather than augment the expected hypercoagulable state in this patient's blood is unclear. The use of aprotinin and other more specific serine protease inhibitors may help to elucidate further the role of the cellular and humoral contributors to hypercoagulable states. From this case, we feel that it is safe to give large-dose aprotinin therapy to patients with factor V Leiden when clinically indicated.

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