Deficiency of Plasma Plasminogen Activator Inhibitor 1 Results in Hyperfibrinolytic Bleeding

By Myoung H. Lee, Evan Vosburgh, Kristen Anderson, and Jan McDonagh

A 63-year-old man was evaluated for a lifelong history of bleeding commencing with frequent epistaxis as a child; all previous routine coagulation parameters were within the normal range. The patient’s hemorrhagic disorder is characterized predominantly by delayed bleeding at surgical sites. In the resting state, there was no clinical or laboratory evidence of excessive fibrinogenolysis. Bleeding was not caused by disseminated intravascular coagulation, factor XIII deficiency, α2-antiplasmin deficiency, or dysfibrinogenemia. It was found that the patient was deficient in plasma PAI-1 antigen and activity but with approximately half normal antigen and normal activity of platelet PAI-1. The low concentration of plasma PAI-1 was insufficient to neutralize circulating t-PA, resulting in high t-PA activity with normal antigen and causing the hyperfibrinolytic activity observed. Studies on seven family members of the proband indicated autosomal inheritance of plasma PAI-1 deficiency. Studies on this patient emphasize a clear correlation between decreased plasma PAI-1 activity and hyperfibrinolytic bleeding and also emphasize the unique role of plasma PAI-1 in the balance between the coagulation and fibrinolytic mechanisms.

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VASCULAR FIBRINOLYSIS is a complex and critical process for controlling hemostasis and thrombosis. It is precisely regulated and is initiated by the release of active tissue plasminogen activator (t-PA) from endothelial cells. t-PA activity is very low in normal blood, and the majority of t-PA is complexed with type 1 plasminogen activator inhibitor (PAI-1). PAI-1 is a relatively unstable molecule with two different conformational forms—active and latent. Binding of PAI-1 to vitronectin stabilizes the active conformation. PAI-1 is in human plasma, platelets, and basement membrane. At present, the origin of plasma PAI-1 is not known, but it is most likely that endothelial cells and/or liver are responsible for plasma PAI-1 production. Platelet PAI-1 is present in α granules.

Recently, much attention has been given to the role of PAI-1 in the fibrinolytic system, suggesting that it is the primary regulatory element in this system. It inhibits both t-PA and urokinase activity by forming a stoichiometric complex with either activator. Computer-simulated kinetic models for the active t-PA levels in plasma predicted that the level of active PAI-1 in the blood is an important regulator of the concentration, half-life, and circadian variation of active t-PA.

Several pathophysiologic problems are reported in relation to abnormal concentrations of plasma PAI-1. Increased PAI-1 activity in blood appears to be associated with certain thromboembolic diseases, such as myocardial infarction, deep vein thrombosis, and postoperative thrombosis in patients undergoing elective hip surgery. However, direct evidence to support the conclusion that elevated PAI-1 activity in blood is a major cause of thrombosis remains to be verified because a 10-fold increase in plasma PAI-1 occurs during pregnancy and is not strongly associated with thromboembolic diseases.

There may be a convincing, if limited, correlation between decreased PAI-1 activity and a hyperfibrinolytic bleeding tendency. Hyperfibrinolytic bleeding is characterized by normal platelet and coagulation activities. Delayed bleeding occurs because a normal hemostatic plug is formed, but it is later more susceptible to breakdown owing to increased fibrinolytic activity. Presently, there are few dissimilar cases reported relating decreased PAI-1 activity to hyperfibrinolytic bleeding. DiCval et al reported a case of a lifelong delayed bleeding disorder associated with a quantitative deficiency of PAI-1 in their patient’s plasma but with a normal level of PAI-1 in platelets; Francis et al described a patient with amyloidosis who had severe bleeding and produced autoantibodies against PAI-1. A patient with low PAI-1 activity and normal PAI-1 antigen was reported by Schleef et al; and a postoperative bleeding case associated with an excess of t-PA activity and low PAI-1 activity was reported by Stankiewicz et al. Fay et al recently reported a case of deficient PAI-1 in plasma and platelets that was explained by a molecular defect in the PAI-1 gene.

In this report, we present a patient with low plasma levels of PAI-1 antigen and activity but with functional platelet PAI-1, which was associated with a lifelong delayed bleeding disorder. We also report plasma PAI-1, t-PA activity, and antigen levels in the patient’s family members.

MATERIALS AND METHODS

Blood sampling and sample preparations. After the patient had rested for 15 minutes, blood was collected into Vacutainer tubes (Becton Dickinson, Rutherford, NJ) containing sodium citrate. Blood was centrifuged (2,000g; 30 minutes) to prepare platelet free plasma for PAI-1 antigen and activity and t-PA antigen measurements. For t-PA activity measurement, blood was immediately acidified and centrifuged (2,000g; 20 minutes) to prepare acidified plasma, followed by activity determinations (COA-SET t-PA; Kabi Diagnostica, Franklin, OH).

Serum was prepared from blood, collected into Vacutainer tubes without anticoagulant. It was incubated at room temperature for 30 minutes, blood was collected into Vacutainer tubes containing sodium citrate. Blood was centrifuged and the supernatant was collected and acidified. The platelet-poor plasma was prepared by centrifugation at 3,000 rpm for 20 minutes.

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2357
minutes, and the clot was removed by centrifugation (2,000g, 20 minutes). Serum was used to measure PAI-1 activity and antigen secreted from the platelets.

**Fibrinogen assays.** Patient's and normal fibrinogens were prepared, and fibrinopeptide release was determined on a C1r-high-pressure liquid chromatograph (HPLC) as previously described. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli. Fibrin degradation products were measured on serum by latex agglutination (Thrombo-Wellcotest; Wellcome, Triangle Park, NC), and D-dimer was also measured by latex agglutination (D-dimer test; Diagnostica Stago, Asnières, France).

**t-PA and PAI-1 measurements.** t-PA activity (COA-SET t-PA, Kabi Diagnostica) and PAI-1 activity (COA TEST PAI; Kabi Diagnostica) were measured with chromogenic assays. PAI-1 activity is expressed as arbitrary units (AU). One AU of PAI-1 is the amount that inhibits 1 U of t-PA during a 10-minute incubation of samples with standard t-PA under experimental conditions (COA TEST PAI; Kabi Diagnostica). t-PA antigen was measured as total t-PA antigen by an enzyme immunoassay, using a monoclonal antibody that measures free t-PA and t-PA-PAI-1 complex (Corvas, San Diego, CA). This assay is reactive to both one- and two-chain t-PA. PAI-1 antigen was also measured by an enzyme immunoassay, either by TimElize PAI-1 (Biopool, Hamilton, Ontario, Canada) or by Imubind PAI-1 (American Diagnostica, Greenwich, CT). In these assays, active, latent and complexed forms of PAI-1 had the same response, according to the manufacturers; so, these assays measured total PAI-1 antigen.

**RESULTS**

**Case report.** The patient is a 63-year-old man with a history of bleeding commencing with frequent episodes of epistaxis as a child. No complications were reported at birth. At age 18, he bled for 1 month beginning the day after dental extractions and for 1 week beginning the day after tonsillectomy. Three inguinal hernia operations (ages 21 to 26) were characterized by normal hemostasis on the day of surgery, followed by hematoma formation and bleeding from the incision for up to 1 month. At age 52, the patient had hematuria for 1 month after a transurethral prostate resection. At age 57, inguinal surgery was again complicated by delayed bleeding, which promptly stopped when he was administered fresh frozen plasma. The patient has frequent and significant bruising but no spontaneous hemarthrosis or hematomas. He has had numerous hemostatic evaluations and was once told that he had factor V deficiency. His family history is significant in that both of his brothers reported some excessive delayed bleeding after dental extractions. His physical examination was normal except for a Marie-Charot-Tooth neuropathy. Clinical test results are summarized in Table 1. All the coagulation parameters appeared to be within the normal range except for the thrombin time and the reptilase time, which were in the upper limit of normal range or slightly prolonged. This originally led us to investigate the possibility of abnormal fibrinogen. Also, the von Willebrand factor (vWF) antigen and cofactor activity were just below the normal range.

**Patient fibrinogen and fibrinopeptide.** Patient fibrinogen was purified in the normal manner from fresh frozen plasma by passage over gelatin-Sepharose and lysine-Sepharose affinity columns followed by repeated (NH₄)₂SO₄ precipitation. The SDS-PAGE result is shown in Fig 1. Surprisingly, the molecular weight of the patient's fibrinogen was lower (~200 Kd) than the control fibrinogen (340 Kd) prepared at the same time. Total fibrinopeptides released from the patient's fibrinogen compared with the control fibrinogen are shown in Fig 2 (A and B). Only about 20% of fibrinopeptide B was released compared with fibrinopeptide A in the patient's fibrinogen, whereas in the control fibrinogen, both fibrinopeptides A and B were completely released. It appeared that the fibrinogen isolated from the patient was a degradation product (mostly fragment X) of native fibrinogen (Fig 1). However, a fast and crude preparation of patient's fibrinogen by 25% (NH₄)₂SO₄ precipitation only, twice in 1 hour, resulted in intact fibrinogen, with complete fibrinopeptide A and B release after thrombin treatment (Fig 2C). In this experiment, the fibrinogen was purified and analyzed approximately 2 hours after the blood was drawn. This finding indicated that the circulating fibrinogen was normal but that this patient had hyperfibrinolytic activity in his plasma. Presumably, the fibrinolytic system was activated in this case by blood collection or in the initial steps of purification.

**Plasma t-PA and PAI-1 activity and antigen level.** To identify the cause of this hyperfibrinolytic activity in the patient, we measured plasma levels of t-PA and PAI-1. Because t-PA and PAI-1 are diurnally fluctuating proteins, blood samples were drawn around 2:00 PM, along with 20 healthy normal controls, 20 to 67 years of age. The average of four determinations of the activities of both t-PA and PAI-1 and the antigen levels of t-PA and PAI-1 are shown in Table 2. t-PA activity in the patient's plasma (1.9 ± 0.1 U/mL) was greater than that of the control (0.9 ± 0.3 U/mL), whereas the t-PA antigen level in the patient's plasma (5.2 ± 0.2) was the same as the control group (4.9 ± 2.0). PAI-1 activity in

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**Table 1. Clinical Test Results**

<table>
<thead>
<tr>
<th>Test</th>
<th>Proband</th>
<th>Normal</th>
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<tbody>
<tr>
<td>PT (s.)</td>
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<td>11-13</td>
</tr>
<tr>
<td>PTT (s.)</td>
<td>36.3</td>
<td>30-37</td>
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<tr>
<td>Factor VIII (%)</td>
<td>67</td>
<td>51-150</td>
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<td>vWF:Ag (U/mL)</td>
<td>0.5</td>
<td>0.6-1.2</td>
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<tr>
<td>vWF:R-Co (U/mL)</td>
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<td>0.6-1.2</td>
</tr>
<tr>
<td>vWF-multimer pattern by 2-D agarose gel</td>
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<td></td>
</tr>
<tr>
<td>Factor V (%)</td>
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<td>75-100</td>
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<tr>
<td>Thrombin time (s.)</td>
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<td>16.0-20.0</td>
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<tr>
<td>Reptilase time (s.)</td>
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<tr>
<td>Fibrinogen (mg/dL)</td>
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</tr>
<tr>
<td>Functional</td>
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<tr>
<td>Factor XIII (%)</td>
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<td>1:1 mix</td>
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<td>Fragment,1,2 (pmol/L)</td>
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<td>D-D (ng/mL)</td>
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<td>α2-Antiplasmin (%)</td>
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<td>Plasminogen (%)</td>
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<td>Platelet count (per μL)</td>
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<tr>
<td>Bleeding time (min)</td>
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<td>2-9</td>
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</table>

* Bleeding recurred 16 hours later.
in platelets is reported to be much higher (270.3 ± 71.9 ng/mL) than in plasma (21.0 ± 7.2 ng/mL), but platelet PAI-1 exists primarily in an inactive, latent form, with only 3% to 4% being active compared with the plasma PAI-1.[9,25] As would be expected, antigen levels in the patient’s serum (111 ± 16 ng/mL) and the control group (229 ± 25 ng/mL) were considerably higher than in plasma. PAI-1 antigen in the patient’s platelets was approximately half (with a normal platelet count, 204,000/μL) compared with the control, but it appeared to be released normally on platelet aggregation. The PAI-1 activity of patient’s serum (6.7 ± 1.5) and control (11.4 ± 3.0) were significantly higher than the values of plasma (Table 3). The difference in PAI-1 activity in the patient’s
serum and plasma was similar to that of controls, ~6 AU/mL. This finding indicated that PAL1 in the patient's platelets was released normally on platelet aggregation, although the concentration in platelets was approximately half of the control.

**t-PA and PAL1 in the patient's family members' plasma.** We also measured the proband's six available family members' plasma t-PA and PAL1 activity and antigen levels on two occasions. Blood was drawn in the afternoon after a 15-minute interval of rest, and measurements were done immediately for PAL1 and t-PA activity. PAL1 and t-PA antigen were measured the next day on plasma kept at 4°C. The test results along with the pedigree of the patient's family are shown in Fig 3. The proband's three siblings had somewhat low levels of PAL1 activity (below 1 SD from the mean). His sister probably would have no detectable PAL1 activity with repeated testing. At the same time, all siblings had t-PA activity >2 SD above the mean. The siblings, like the proband, had normal t-PA antigen levels.

**DISCUSSION**

Delayed bleeding disorders can be associated with abnormal fibrinolysis. They also have been associated with deficiencies of several proteins involved in the regulation of fibrinolysis, including α2-antiplasmin,26,27 factor XIII,28,29 and PAL1.17,19 Factor XIIIa regulates fibrinolysis in two ways: by introducing covalent cross-links into fibrin and by cross-linking α2-antiplasmin to fibrin.30 These cross-linking reactions render the fibrin clot more resistant to degradation by plasmin. Factor XIII deficiency is characterized by delayed bleeding because a non-cross-linked clot is formed and is more readily lysed by plasmin. α2-Antiplasmin is the primary inhibitor of plasmin activity in blood. α2-Antiplasmin deficiency is also characterized by delayed bleeding; in this case because a normal clot forms, but it then lysed readily because there is no inhibitor to abate plasmin activity.

Decreased PAL1 activity may be caused by quantitative defects, qualitative defects, or antibodies against PAL1. There are two documented cases of a quantitative PAL1 deficiency, and both were associated with a lifelong hemorrhagic disorder.17,21 In one case, both PAL1 antigen and activity in the patient's plasma were below the sensitivity of the assays, whereas platelet PAL1 activity and antigen were normal.17 The other case, recently reported by Fay et al,22 had a two-base insertion in the PAL1 gene, which produced a frameshift in exon 4 and led to early truncation of the PAL1 protein with a correspondingly low level of PAL1 antigen in both plasma and platelets. Deficiency in plasma PAL1 caused high t-PA activity, which resulted in a hyperfibrinolytic state. Schleef et al reported a bleeding diathesis due to decreased functional activity of PAL1.19 Both plasma and platelet PAL1 had low activity with normal antigen level and diminished complex formation with t-PA resulting in hyperfibrinolysis. The exact defect in this patient's PAL1 molecule was not characterized. However, it was suggested that an abnormality could be due to alternative processing of PAL1 mRNA or to a defect in the 12.2-kb PAL1 gene.

Francis et al18 reported a patient with amyloidosis with delayed bleeding and accelerated fibrinolysis in whom there were neutralizing antibodies to PAL1 and a normal t-PA antigen level but high t-PA activity. In 1983, Booth et al reported a case of a lifelong hemorrhagic disorder due to excess plasminogen activator, but the role of PAL1 in this case was not investigated.31

Our patient clearly shows that a quantitative deficiency of plasma PAL1 activity and antigen levels is associated with a lifelong delayed bleeding disorder. The decrease in plasma PAL1 results in an elevation of the free t-PA activity in plasma, which in this patient was calculated to be ~5 ng/mL. The total t-PA concentration in this patient's plasma was determined to be 8 ng/mL, which is comparable with the calculated free t-PA concentration. Thus, most of the plasma t-PA circulated as a free form with high activity causing a hyperfibrinolytic state in the plasma. Because 93% of the total PAL1-1 antigen in blood is reported to be present in platelets,75 the trace amount of PAL1 sometimes detected in the patient's plasma could have been derived from his platelets or could be due to the vagaries of the assays. His serum had significant levels of PAL1 antigen; approximately half that compared with the control.
The inheritance of congenital PAI-1 deficiency is not clear from the literature or from this case. The proband has a small family living in this country. His siblings seemed to have low PAI-1 activity and high t-PA activity with normal antigen. This would point to this being an inherited characteristic; but at present, these assays cannot define heterozygotes with certainty.

The observation that this patient had a quantitative deficiency of plasma PAI-1 but normal functional platelet PAI-1 suggests the importance of compartmentalization of PAI-1. Because platelets have approximately 10 times more PAI-1 antigen than the circulating plasma, and because platelet PAI-1 is released after platelet activation, it is not obvious why a deficiency in the plasma compartment only could result in serious bleeding. Simpson et al reported that the plasma and platelet pools of PAI-1 vary independently in various disease states, and the two pools of PAI-1 do not seem to mix. It may be that plasma PAI-1 is responsible for immediate complex formation with circulating t-PA. The primary function of the platelet compartment of PAI-1 is thought to be a major determinant for clot-lysis resistance and to be associated with thrombosis resistance. It may protect the blood clot against premature lysis during coagulation, but it is not available at later times when delayed bleeding might occur. However, Kunitada et al recently showed that although platelets impair clot lysis by t-PA in vitro, this is not owing to inactivation by PAI-1 but to clot retraction and decreased binding of the plasminogen activator. One can speculate that the precise local concentrations of PAI-1 and the dynamic time course are important in controlling hemorrhage. This patient emphasizes the importance of the role of plasma PAI-1 in some hyperfibrinolytic bleeding disorders.

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