A Lifelong Bleeding Disorder Associated With a Deficiency of Plasminogen Activator Inhibitor Type 1

By Jocelyne Dieval, Geneviève Nguyen, Sylvie Gross, Jacques Delobel, and Egbert K.O. Kruithof

A 36-year-old patient was investigated for a lifelong history of epistaxis and delayed bleeding after minor surgeries. Deficiencies or abnormalities of the coagulation system, of platelet function, or of factor XIII and α-2-antiplasmin were excluded. Consistently, however, over a period of 7 years, a high basal euglobulin fibrinolytic activity was observed that was characterized by a high tissue-type plasminogen activator (t-PA) activity, normal t-PA antigen, and undetectable plasminogen activator inhibitor type-1 (PAI-1) antigen and activity. The high specific activity of t-PA (640,000 IU/mg) and the minimal amounts of t-PA/PAI-1 complexes detected by fibrin zymography suggest that in this patient all t-PA was active. This is in striking contrast to normal plasma, where the majority of t-PA is complexed to PAI-1. Thus, in this patient, a severe deficiency of PAI-1 is associated with a delayed type bleeding tendency. Our observation underscores the importance of plasma PAI-1 for the stabilization of the hemostatic plug.

HEMOSTASIS, the complex interplay between platelet aggregation, coagulation, and fibrinolysis, is a vital defense system that rapidly stops hemorrhage in case of injury. Excessive bleeding or thrombotic complications are avoided by a precise and sequential regulation of the diverse hemostasis subsystems. Hyperfibrinolytic hemorrhage is characterized by a normal primary hemostasis, but bleeding typically resumes after a delay of up to a few days. Plasmin, the central enzyme of the fibrinolytic system, is generated from its precursor, plasminogen, through the action of plasminogen activators (PAs), of which two distinct types have been identified: tissue-type PA (t-PA) and urinary-type PA (u-PA). The rate of fibrin degradation is regulated, in part, by the specific inhibitors, α-2-antiplasmin and PA-inhibitor type 1 (PAI-1). Hyperfibrinolytic bleeding caused by a deficiency of α-2-antiplasmin has been well documented. The role of PAI-1 in the protection of newly formed fibrin from premature lysis is still not well established. Rather, high levels of PAI-1 have been associated with thrombophilia, an increased risk of reinfarction in survivors of myocardial infarction, and postoperative thrombosis in patients undergoing elective hip surgery. Recently, Schleef et al described a patient with a bleeding tendency associated with normal PAI-1 antigen but low activity, and Francis et al described a patient with severe bleeding and a monoclonal antibody to PAI-1.

We report here a case of a severe quantitative PAI-1 deficiency associated with a lifelong tendency to delayed, recurrent bleeding in a 36-year-old man.

PATIENT AND METHODS

Patient. The patient, born in 1953, first presented in 1982 for an important hemorrhhiasis after meniscectomy. He had a lifelong history of epistaxis and delayed bleeding after surgery or trauma (eg, after an amygdalectomy, an appendectomy, and a head injury). He also experienced a hematuria 2 days after a renal contusion. After two dental extractions, bleeding stopped rapidly but resumed 24 hours later, and, on one occasion, dental extraction was complicated by a sublingual hematoma. None of these hemorrhagic episodes required blood transfusion, and on all occasions bleeding stopped spontaneously. Physical examination of the patient showed no particular abnormalities. He had a normal cardiovascular function and no hepatosplenomegaly or clinical evidence of liver disease. After the diagnosis of a hyperfibrinolytic state was made, the patient was treated with epsilon aminocaproic acid before other dental extractions, and in contrast to previous minor traumas, no delayed bleeding was noted. The patient has given written consent to participate in the study.

Hemostasis tests. Blood was collected at 10 AM and 4 PM, after a 15-minute rest period and anticoagulated with 0.13 mol/L of sodium citrate (9 vol:1 vol). Platelet-poor plasma was obtained after 15 minutes of centrifugation at 3,500g. For the study of the fibrinolytic parameters, blood was drawn before and after a 10-minute venous stasis (cuff pressure between the systolic and the diastolic pressures), and immediately cooled in melting ice. The coagulation tests and the platelet function tests were performed by standard techniques. Plasminogen antigen was measured by radial immunodiffusion. Plasminogen and α-2-antiplasmin activities were measured by spectrophotometric assays (Behring, Marburg, Germany), and plasmin–α-2-antiplasmin complexes by crossed immunoelectrophoresis. Factor XIII activity was measured by a coagulation assay (Behring) and fibrin degradation products by a latex immunnoassay (D-dimer test; Diagnostica Stago, Asnières, France). β-Thromboglobulin was measured by enzyme-linked immunosorbent assay (ELISA) (Asserachrom βTG, Diagnostica Stago). Euglobulin precipitation was performed by diluting the plasma 10-fold in cold distilled water and adjusting the pH to 5.9 with 0.25% acetic acid. The euglobulins were dissolved in 0.1 mol/L Tris HCl buffer, pH 7.5 and their fibrinolytic activity measured by the euglobulin clot lysis time assay (ECLT). t-PA antigen was measured by ELISA (Tint Elize t-PA; Biopool, Umeå, Sweden) and t-PA activity with a chromogenic assay (Spectrolyse Fibrin; Biopool). PAI-1 antigen was measured by radioimmunoassay (RIA) and ELISA (Tint Elize PAI-1; Biopool), and PAI activity with a chromogenic substrate assay (Spectrolyse Fibrin; Biopool). Fibrin zymography was performed on a plasminogen-rich fibrin

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To identify the bands on the zymography, the euglobulins were incubated with rabbit antiserum to t-PA, PAI-1, u-PA, or α-2-antiplasmin, for 2 hours at 37°C, and the immune complexes precipitated using cellulose coated with donkey antirabbit antibodies (Sac antisera to t-PA, PAI-1, u-PA, or cy-2-antiplasmin, for 2 hours at 3°C.

To identify the bands on the zymography, the euglobulins were incubated with rabbit antihuman PAI-1 and antihuman t-PA. When the euglobulins of the patient were incubated with rabbit antihuman PAI-1 or antihuman t-PA, the intensity of the α-2-antiplasmin band increased threefold, but a large band of free t-PA became detectable. In contrast, the patient zymography showed a faint band at the 110-Kd position only when euglobulins were concentrated fourfold, but a large band of free t-PA was already visible in unconcentrated euglobulins. Thus, in the patient PAL1 levels are much lower than in the low PAI-1 control. The identity of the 110-Kd band as the complex of t-PA with PAI-1 was demonstrated by immunoprecipitation with rabbit antihuman PAI-1 and antihuman t-PA. When the euglobulins of the patient were incubated with rabbit antihuman PAI-1 or antihuman t-PA, the intensity of the 110-Kd band decreased as shown in Fig 2, but not with rabbit antisera against human α-2-antiplasmin. Because platelets also contain PAI-1, we tried to estimate the contribution of platelets to the PAI-1 measured in the patient plasma. The plasma level of β-thromboglobulin (23 ng/mL) suggests that the patient plasma contains between 0.2 and 0.5 ng/mL of platelet-derived PAI-1 (ratio PAI-1/β-thromboglobulin = 0.01 to 0.02)."

**Table 1. Coagulation Parameters in the Patient With Bleeding and Deficient PAI-1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding time (Ivy method, min)</td>
<td>5</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Platelet count (G/L)</td>
<td>23</td>
<td>1.5-4</td>
</tr>
<tr>
<td>Prothrombin time (s)</td>
<td>12</td>
<td>11-13</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>34</td>
<td>30-37</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>1.7</td>
<td>1.8-4</td>
</tr>
<tr>
<td>Thrombin time (s)</td>
<td>17</td>
<td>16-20</td>
</tr>
<tr>
<td>Factor XII (IU/mL)</td>
<td>1.0</td>
<td>0.7-1.5</td>
</tr>
</tbody>
</table>

**Table 2. Fibrinolytic Parameters in the Patient With Bleeding and Deficient PAI-1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECLT (min)</td>
<td>Before stasis 80-105 After stasis 10-15</td>
<td>&gt;140 10-90</td>
</tr>
<tr>
<td>t-PA antigen (ng/mL)</td>
<td>Before stasis 5 After stasis 3-13</td>
<td>3-13</td>
</tr>
<tr>
<td>t-PA activity (IU/mL)</td>
<td>Before stasis 3.2 After stasis 2.0-2</td>
<td>0.2-2</td>
</tr>
<tr>
<td>PAI-1Ag (ng/mL) ELISA</td>
<td>Before stasis &lt;2 After stasis 61</td>
<td>12.5-39</td>
</tr>
<tr>
<td>PAI activity (U/mL)</td>
<td>Before stasis &lt;1 After stasis 4.8-17.2</td>
<td>4.8-17.2</td>
</tr>
<tr>
<td>α-2-antiplasmin (%)</td>
<td>100</td>
<td>80-120</td>
</tr>
<tr>
<td>Plasminogen antigen (g/L) activity (%)</td>
<td>0.1</td>
<td>0.1-0.2</td>
</tr>
<tr>
<td>Fibrin degradation products (μg/mL)</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

*Lower limit of detection for PAI-1 Ag by ELISA method is 2 ng/mL, and by RIA (1) is 6 ng/mL.

†The detection limit for PAI activity is 1 U/mL. The patient PAI1 antigen and activity were measured at 10 AM and 4 PM.

Abbreviation: APTT, activated partial thromboplastin time.
94-

from a control subject with low PAL1 antigen and activity (3.5 ng/mL
twofold
67-
ysis, followed by zymography on a plasminogen-rich fibrin agarose
underlay. The zymographies were incubated for 48 hours at 37°C.
(Lanes A through C) Patient euglobulins concentrated fourfold (A),
dilutions of the euglobulins were made in the same buffer. The
different dilutions of the euglobulins were analyzed by electrophore-
sis, followed by zymography on a plasminogen-rich fibrin agarose
panel A through C). Patient euglobulins concentrated fourfold (A),
twofold (B), and unconcentrated (C). (Lanes D through G) Euglobulins
from a control subject with low PAI-1 antigen and activity (3.5 ng/mL
by ELISA and 1.5 U/mL) diluted twofold (D), unconcentrated (E),
concentrated twofold (F), and concentrated fourfold (G). The molecu-
lar weight markers are given in kilodaltons. The control (t-PA:
Ag = 6.0 ng/mL) showed mainly a 110-Kd band, whereas the patient
(t-PA:Ag = 5.2 ng/mL) showed mainly a band at 67 Kd.

DISCUSSION

Deficiencies of the fibrinolytic system sometimes may be
responsible for severe, lifelong bleeding disorders. The
hemorrhagic consequences of deficiencies of α-2-
antiplasmin and of factor XIII, the factor that cross-links
α-2-antiplasmin to fibrin and renders the fibrin more
resistant to fibrinolysis, are well known, whereas those of
PAI-1 deficiencies still need to be established. In typical
hemorrhagic states caused by deficiencies in α-2-
antiplasmin and of factor XIII, platelet activation and
aggregation are not affected, and fibrin polymerization
takes place normally, but the fibrin is more sensitive to the
action of plasmin because the fibrin is not stabilized by
factor XIII and no α-2-antiplasmin is adsorbed on the clot.
Therefore, primary hemostasis explored by the bleeding
time is normal as in our patient, but the clot dissolution by
physiologic fibrinolysis is accelerated leading to sometimes
excessive and prolonged bleeding after a delay of up to
several days. The characteristics of hyperfibrinolytic bleed-
ing (ie, a normal primary hemostasis followed, after a delay
of up to several days, by sometimes excessive and prolonged
bleeding) were also observed in our patient. The diagnosis
of constitutive hyperfibrinolysis because of a deficiency of
plasma PAI-1 is based on the following observations: (1)
The patient has a lifelong history of delayed bleeding after
minor trauma and we were unable to detect any abnormal-
ity of coagulation or platelet function. The slight reduction
of fibrinogen is clearly insufficient to account for the
bleeding tendency. (2) A high fibrinolytic activity was
measured on five occasions over a period of 7 years. (3)
Both PAI-1 antigen and PAI-1 activity were undetectable in
the patient’s plasma. However, PAI-1 levels in serum were
normal, indicating that the deficiency is limited to the
plasma compartment. (4) A possible inversion of the
diurnal variation of PAI-1 was excluded. (5) The specific
activity of t-PA in the patient at rest was 640,000 IU/mg, a
value similar to the international t-PA standard. This result
in combination with that of fibrin-zymography demonstra-
ates that in the patient plasma all t-PA is active. In
contrast, in similarly collected plasmas of patients and
controls (ie, resting, morning plasma and no precaution
taken to prevent the in vitro reaction of t-PA with PAI-1)
the specific activity of t-PA is very low and the majority of
t-PA is complexed to PAI-1. (6) Treatment with EACA,
the treatment of choice in hyperfibrinolytic bleeding, com-
pletely prevented bleeding after dental extractions, whereas
on previous occasions delayed bleeding occurred. Several
reports of hyperfibrinolytic bleeding because of uncon-
trolled t-PA activity have appeared in recent years. Booth
et al described a patient with a lifelong bleeding disorder
caused by excessive t-PA activity. Whether the excessive
activity was due to high t-PA levels or an absence of PAI-1
was not established. Liebman et al observed a bleeding
disorder and increased fibrinolysis in amyloidosis. In recent
preliminary reports, excessive fibrinolytic activity associated
with delayed bleeding was attributed to high t-PA antigen
levels, but normal PAI-1, in one patient and to a mono-
clonal, amyloidogenic autoantibody to PAI-1 in another.

Fig 1. Zymography of the euglobulins of the patient with PAI-1
deficiency. Euglobulin precipitates obtained from 1 mL of citrated
plasma were dissolved in 250 μL of 0.1 mol/L Tris-HCl buffer pH 7.5,
resulting in a fourfold concentrated euglobulin fraction. Further
dilutions of the euglobulins were made in the same buffer. The
different dilutions of the euglobulins were analyzed by electrophore-
sis, followed by zymography on a plasminogen-rich fibrin agarose
panel A through C). Patient euglobulins concentrated fourfold (A),
twofold (B), and unconcentrated (C). (Lanes D through G) Euglobulins
from a control subject with low PAI-1 antigen and activity (3.5 ng/mL
by ELISA and 1.5 U/mL) diluted twofold (D), unconcentrated (E),
concentrated twofold (F), and concentrated fourfold (G). The molecu-
lar weight markers are given in kilodaltons. The control (t-PA:
Ag = 6.0 ng/mL) showed mainly a 110-Kd band, whereas the patient
(t-PA:Ag = 5.2 ng/mL) showed mainly a band at 67 Kd.

Fig 2. Identification of the lysis bands in the zymography of the
patient. Euglobulins were incubated for 2 hours at 37°C with rabbit
antisera to human PAI-1, t-PA, u-PA, and α-2-antiplasmin. The immune
complexes were precipitated using cellulose coated with donkey
antirabbit antibodies. After centrifugation, the supernatants were
analyzed by sodium dodecyl sulfate 7.5% polyacrylamide gel electro-
phoresis followed by zymography for 96 hours. (Lane A), Rabbit
gamma globulins; (lane B), anti-α-2-antiplasmin; (lane C), anti-u-PA;
(lane D), anti-t-PA; (lane E), anti-PAI-1; (lane F), no antisera. The
molecular mass markers are given in kilodaltons. The decrease in the
intensity of the 67-Kd band in lane D only, and of the 110-Kd band in
lanes D and E identifies the 67-Kd band as free t-PA and the 110-Kd
band as a t-PA/PAI-1 complex.
In a 76-year-old patient with a lifelong bleeding tendency, high fibrinolytic activity was attributed to a functionally abnormal PAI-1.

Our patient represents the first case of a quantitative deficiency of plasma PAI-1. Traces of PAI-1 were detected in the patient plasma, but they may have been derived from the patient platelets. Indeed, the patient plasma contained 23 ng/mL of β-thromboglobulin and because the platelets of the patient contained normal amounts of PAI-1, we can estimate the amount of platelet PAI-1 that should be present in the plasma. Using a ratio of PAI-1/β-thromboglobulin of 0.01 to 0.02, we may estimate that between 0.2 and 0.5 ng/mL of PAI-1 in the plasma is platelet derived. Because the concentration of PAI-1 detected in the plasma of our patient was much lower than 2 ng/mL, it is even possible that all the PAI-1 detected in the plasma is platelet derived. The observation of normal levels of PAI-1 antigen and activity in the serum suggests that there are no major abnormalities of the PAI-1 gene. Because platelet PAI-1 is stored intracellularly, it is possible that the deficiency is at the level of secretion, as has been described for the Z variant of α-1-antitrypsin deficiency. It is also possible that the tissue-specific expression of PAI-1 in the cells contributing to plasma PAI-1 (endothelial cells and possibly also hepatocytes) is abnormal.

The role of PAI-1 in the stabilization of the primary hemostatic plug is not yet well established. It is known that PAI-1 is the primary inhibitor of t-PA and urokinase in plasma, and that the release of PAI-1 by aggregating platelets may provide a means to prevent premature fibrinolysis and thus contribute to the stabilization of the hemostatic plug in the early stage of hemostasis. In addition, recent studies have shown that PAI-1 binds to the fibrin clot, directly or via the vitronectin that is also secreted by the platelets during their activation, and fibrin-bound PAI-1 was still able to inhibit t-PA and u-PA activities. t-PA/PAI-1 complexes compete with free t-PA for fibrin binding, thus reducing the amount of free t-PA adsorbed on fibrin and, therefore, its fibrinolytic activity.

Because platelet PAI-1 content and secretion appear to be normal in our patient, the association of a quantitative deficiency of plasma PAI-1 with a bleeding disorder suggests that plasma PAI-1 is essential for the maintenance of the integrity of the hemostatic plug. It is not known whether the good response to venous stasis of the patient has also contributed to the bleeding tendency.

We conclude that in our patient, the deficiency of plasma PAI-1 associated with a high potential release of t-PA led to uncontrolled t-PA activity on fibrin clots. This observation emphasizes the importance of plasma PAI-1 in the stabilization of the hemostatic plugs, and provides the first documented demonstration of the importance of PAI-1 in the control of the in vivo fibrinolysis.

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A lifelong bleeding disorder associated with a deficiency of plasminogen activator inhibitor type 1

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