Stroke in a Young Adult With Familial Plasminogen Disorder

Anthony J. Furlan, MD; Fred V. Lucas, MD; Romeu Craciun, MD; and Robert C. Wohl, PhD

Background: We report a new plasminogen disorder detected in a 29-year-old man with a cerebellar infarct. To our knowledge, plasminogen disorders have not been previously linked with stroke.

Summary of Report: Tests for well-recognized causes of stroke were negative. However, a screening hypercoagulation profile indicated low functional levels of plasminogen activity. Immunologic plasminogen (Laurell technique) was 64% of normal (normal level, 80–130%). The rate of plasmin generation induced by adding urokinase to plasma was also low. Plasminogen activator, free protease, and α2-plasmin inhibitor levels were normal. Family studies detected a similar plasminogen abnormality in the patient’s mother and 9-year-old son, both of whom are asymptomatic.

Conclusions: Our patient shows a congenital, heterozygous, functionally abnormal plasminogen. Although the exact relationship to stroke is unclear, we suggest screening young patients with unexplained stroke for plasminogen defects using commercially available assay systems. (Stroke 1991;22:1598-1602)

Abnormal variants of plasminogen have rarely been described. Aoki et al,1 Soria et al,2 and others3,4 have described families with normal plasminogen antigen levels but defective functional activity due to active site defects. Wohl et al5-7 studied three unrelated individuals, two of whom exhibited impaired activator binding and one who exhibited combined impairment of activator binding and defective cleavage of the Arg560-Val bond that converts plasminogen to plasmin. These abnormalities have all been detected as a result of investigation of patients with deep vein thrombosis. Subsequently, family studies have disclosed relatives with the molecular abnormality who lack a history of thrombosis.

Schutta et al8 recently described a patient with increased intracranial pressure caused by cerebral venous thrombosis and plasminogen deficiency. However, inherited abnormalities of plasminogen have not been previously associated with stroke. In this report, we describe an abnormal plasminogen in a young man who presented to us for a cerebellar infarct. The plasminogen abnormality has subsequently been detected in his son and his mother, both of whom are asymptomatic.

Case History

The patient is a 29-year-old man who suffered a left cerebellar infarct and was referred to the Cleveland Clinic for evaluation. There was no history of cigarette smoking, migraine headaches, hypertension, cardiac disease, drug abuse, or other major stroke risk factors. There was no family history of thrombotic events. Physical examination showed mild left cerebellar signs, but the patient was otherwise normal. A digital subtraction angiogram of the aortic arch and extracranial vessels showed no occlusive lesions. A sector computed tomography scan of the posterior fossa revealed a 1x2.5-cm area of decreased attenuation in the left cerebellar hemisphere consistent with an infarct in the distribution of the left superior cerebellar artery. Tests for vasculitis, cardiac disease, platelet function, antithrombin III level, fibrinogen, lupus anticoagulant, and other known causes of stroke were normal or negative. However, a screening coagulation profile indicated decreased plasminogen activity.

Methods

Screening assays of plasminogen and α2-plasmin inhibitor in plasma were performed using the D-val-leu-lys aminoisophthalic acid dimethyl ester fluorescent substrate and the Protopath instrument (Amer-
TABLE 1. Results of Screening Coagulation Studies in Patient Compared With Normal Values

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated whole blood clotting time</td>
<td>135 sec</td>
<td>90–130</td>
</tr>
<tr>
<td>Circulating anticoagulant</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Platelet count</td>
<td>319 x 10^9/l</td>
<td>150–400 x 10^9/l</td>
</tr>
<tr>
<td>Activated partial thromboplastin time</td>
<td>35 sec</td>
<td>21–31 sec</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>13 sec</td>
<td>10–13 sec</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>13 sec</td>
<td>10–13 sec</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombin clottable</td>
<td>182 mg/dl</td>
<td>200–400 mg/dl</td>
</tr>
<tr>
<td>Radial immunodiffusion</td>
<td>210 mg/dl</td>
<td>200–400 mg/dl</td>
</tr>
<tr>
<td>Antithrombin III, plasma</td>
<td>120%</td>
<td>80–120%</td>
</tr>
<tr>
<td>(D-phe-pro-arg-AIE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet aggregation ADP, epinephrine, collagen, arachidonic acid, ristocetin</td>
<td>Normal dose responses</td>
<td></td>
</tr>
<tr>
<td>α-Plasmin inhibitor</td>
<td>91%</td>
<td>80–120%</td>
</tr>
<tr>
<td>(D-val-leu-lys-AIE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasminogen (D-val-leu-lys-AIE)</td>
<td>1.1 CTA units/ml</td>
<td>2.4–3.8 CTA units/ml</td>
</tr>
</tbody>
</table>

AIE, aminoisophthalic acid dimethyl ester; CTA, commercial thrombolytic agents.

ican Dade, Inc., Miami, Fla.). Platelet-poor plasma was subsequently prepared by centrifugation at 4,000 g of 9 parts venous whole blood, which was collected in plastic syringes containing 1 part 3.8% (wt:vol) sodium citrate. The plasma was either tested immediately or kept at −70°C until further use.

Plasma plasminogen was measured using a 9 M excess of streptokinase over plasminogen and either Na-carbobenzoxy-L-lysine p-nitrophenyl ester or H-D-val-leu-lys-p-nitroanilide as substrates. The plasminogen-streptokinase complex active site concentration was determined by initial velocity measurements using the above substrates in a Beckman 25 recording spectrophotometer and also by p-nitrophenyl p′-guanidinobenzoate titrations. The conditions for these and subsequently described measurements have been previously reported.5-7,9,10

The amount of plasma α2-plasmin inhibitor was based on inhibition of amidolysis of H-D-val-leu-lys-p-nitroanilide using a known amount of purified plasmin. The initial velocity of the reaction using test samples was measured and compared with the control value without plasma.9

Plasmin generation rates in plasma were determined using both urokinase and lower concentrations of streptokinase and the H-D-val-leu-lys-p-nitroanilide substrate. The calculated initial velocity of plasminogen activation in plasma was divided by the calculated initial velocity generated in a pure Glu-plasminogen solution under the same conditions. This ratio represents the observable plasma plasmin generation rate.5,9

Measurements of plasminogen antigen were performed by using a modification of the Laurell rocket immunoelectrophoresis technique.9

Plasma from the patient (100 ml) was processed using L-lysine-sepharose affinity chromatography. Pure native Glu-plasminogen (5.59 mg) was recovered and stored frozen in the presence of 0.2 M ε-amino-n-caproic acid buffer. Absorbance of the pure plasminogen at 280 nm yielded 91% of the values obtained by the corresponding rocket immunoelectrophoresis on the pure plasminogen. Sodium dodecyl sulfate gel electrophoresis confirmed the purity of the plasminogen preparation and its identity as native Glu-plasminogen.

Active site titrations were performed using p-nitrophenyl-p′-guanidinobenzoate.5,11

Kinetic constants ($K_a$ and $K_{cat}$) of amidase activity were calculated for plasmin, plasmin-streptokinase, and plasminogen-streptokinase derived from the purified material using H-D-val-leu-lys-p-nitroanilide as substrate.5-7 All kinetic constants were calculated on the basis of molar concentration of active sites, not on the basis of protein concentration.

Results

Initial screening coagulation studies (Table 1) were entirely normal except for the functional assay for plasminogen, which was decreased to 1.1 CTA units/ml (normal level, 2.4–3.8 CTA units/ml). This
<table>
<thead>
<tr>
<th></th>
<th>Plasminogen</th>
<th>a2-Plasmin inhibitor</th>
<th>Plasmin generation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasmogen, H-D-val-leu-lys-(p)-nitroanilide</td>
<td>ImmunoLogic concentration</td>
<td>Plasmogen, val-leu-lys-(p)-nitroanilide</td>
</tr>
<tr>
<td></td>
<td>Functional concentration</td>
<td>Immunologic concentration</td>
<td>Functional concentration</td>
</tr>
<tr>
<td></td>
<td>D-val-leu-lys-AIE (nl, 2.4–3.8 CTA units/ml)</td>
<td>H-D-val-leu-lys-(p)-nitroanilide (nl, 16.7–23.7 mg/dl)</td>
<td>D-val-leu-lys-AIE (nl, 80–120%)</td>
</tr>
<tr>
<td>Generation I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JB I (father)</td>
<td>3.1</td>
<td>23.2</td>
<td>95</td>
</tr>
<tr>
<td>*HB (mother)</td>
<td>1.8</td>
<td>10.2</td>
<td>59</td>
</tr>
<tr>
<td>Generation II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*JB II (propositus)</td>
<td>1.1</td>
<td>8.4</td>
<td>64</td>
</tr>
<tr>
<td>NK (sister)</td>
<td>4.2</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>JM (sister)</td>
<td>4.0</td>
<td>30.4</td>
<td>...</td>
</tr>
<tr>
<td>Generation III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*JB III (son)</td>
<td>0.92</td>
<td>9.2</td>
<td>50</td>
</tr>
<tr>
<td>MB (daughter)</td>
<td>2.8</td>
<td>16.4</td>
<td>...</td>
</tr>
<tr>
<td>MC (nephew)</td>
<td>3.8</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>CC (niece)</td>
<td>5.2</td>
<td>34.2/20.1</td>
<td>...</td>
</tr>
<tr>
<td>AM (nephew)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>KM (niece)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

AIE, aminoisophthalic acid dimethylester; CTA, commercial thrombolytic agents.

*Abnormal values.
low value was confirmed by repeat determinations 1 month later. Based on these tests, a more thorough examination of the patient's family as well as the molecular nature of this apparent abnormality in plasminogen was conducted.

Figure 1 is a diagram of the patient's family and indicates members who were tested as well as those in whom an abnormality was found. As can be seen, both the patient’s mother and his son showed an abnormality in plasminogen. This pattern of inheritance is consistent with that of codominant alleles. These findings are consistent with those of Aoki et al. Neither of these other affected family members had a history of thrombotic disease.

Table 2 is a compilation of data from various family members. Rate assays of functional plasminogen and plasmin inhibitor concentration were performed using excess streptokinase and H-D-val-leu-lys-p-nitroanilide as the substrate. These generally show good agreement with the fluorogenic method used in the clinical laboratory. Free protease activity levels in these plasma samples were negligible. The rate of plasmin generated in whole plasma using either urokinase or streptokinase as activators was compared with that which is observed using purified plasminogen preparations. This ratio, the plasmin generation rate, was approximately half normal due to an eightfold rise in a second order rate constant (kcat) shows minor and probably not significant variations from normal. The resulting second order rate constant (kcat/Km) is essentially normal except for the plasminogen–streptokinase complex in which it reflects the altered Km.

Table 4 summarizes the kinetic constants of activation of the patient's plasminogen when urokinase and streptokinase are the activators and H-D-val-leu-lys-p-nitroanilide is the plasmin substrate. In these experiments, the kcat is equivalent to the apparent Michaelis constant (Km) for plasmin, plasminogen-streptokinase, in which case it is approximately twice normal. The catalytic rate constant (kcat) is essentially normal except for the plasminogen–streptokinase complex in which it reflects the altered Km.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>K_m (μmol/l)</th>
<th>k_cat (l/sec)</th>
<th>k_cat/K_m (μmol/l)</th>
<th>K_plg</th>
<th>K_plg/K_m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmin</td>
<td>242±10</td>
<td>23.09±0.94</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(normal)</td>
<td>120±10</td>
<td>25.59±1.05</td>
<td>0.12</td>
<td>8.95±0.45</td>
<td>74.6</td>
</tr>
<tr>
<td>Plasmin-streptokinase</td>
<td>682±38</td>
<td>64.7±3.56</td>
<td>0.10</td>
<td>34.83±4.35</td>
<td>37.1</td>
</tr>
<tr>
<td>(normal)</td>
<td>780±70</td>
<td>51.92±2.60</td>
<td>0.07</td>
<td>42.39±3.70</td>
<td>30.3</td>
</tr>
<tr>
<td>Plasminogen-streptokinase</td>
<td>682±72</td>
<td>33.07±3.47</td>
<td>0.05</td>
<td>32.0±0.01</td>
<td>0.12</td>
</tr>
<tr>
<td>(normal)</td>
<td>320±40</td>
<td>39.11±2.60</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

K_m, Michaelis constant; k_cat, catalytic rate constant.

### Discussion

We now report a previously undescribed abnormality of plasminogen, which we have termed Cleveland, in three immediate generations of a family: the patient, his mother, and his 9-year-old son. Based on the family study and the data on the enzymatic properties of these plasminogens, we conclude that this is a congenital, heterozygous abnormal plasminogen. The plasminogen abnormality was detected by a screening hypercoagulation profile in a 29-year-old man with a cerebellar infarct and no other identifiable cause. The patient has had no further events on aspirin during 3 years of follow-up; the family members are clinically silent on no specific therapy.

The plasminogen abnormality is characterized by defective functional activity in the plasma of all three individuals. The plasminogen is not activated...
by urokinase normally, and in the patient and son it
does not complex with streptokinase as well as with
normal. The decreased activity cannot be attributed
to elevated plasmin inhibitor levels, as these were
normal. The abnormality is also characterized by
decreased antigen levels in all three individuals.
The patient's father is totally normal.

Only the activators' much stronger binding affinity
to the abnormal (potentially dead) plasminogen
than to normal plasminogen can explain the plas-
minogen activation kinetics. The binding affinity is
8–10 times stronger than that for normal plasmino-
gen molecules. This heterozygosity in molecular
behavior leads to interference of potentially dead
molecules (urokinase). Meanwhile, streptokinase
activation data shows that the abnormal plasmino-
gen molecules not only interfere with the binding of
plasminogen substrate to the activator complex, but
also in the formation of the activator complex itself.
The abnormality in this family is therefore most
similar to the Chicago III case reported by Wohl et
al.\(^\text{7}\). The most striking difference between the two
reports is that Cleveland has decreased concentra-
tions of antigen in addition to a functional defect.

The association between the stroke and the plas-
minogen abnormality is unclear because the pa-
tient's mother and son are asymptomatic, and plas-
minogen defects are not routinely examined for in
patients with stroke. An increasing number of pre-
 thrombotic states have been linked with stroke, especially in young adults.\(^\text{12}\) These include anti-
 thrombin III, protein C, and protein S deficiencies,
and the lupus anticoagulant syndrome. Further-
more, because the cause of cerebral infarction
remains unclear in 30–50% of young adults with
stroke, any new potential etiology is of some clinical
importance. We suspect that plasminogen disorders
are rare causes of brain infarction. Nonetheless,
most centers routinely screen young patients with
unexplained stroke for coagulopathies and, since
the plasminogen assay is commercially available
and easy to perform, we suggest adding it to the
battery of available screening coagulation studies.

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