Coagulation-Fibrinolysis System in Poststroke Patients Receiving Antiplatelet Medication

Hideo Tohgi, MD; Hiroaki Takahashi, MD; Kenichi Chiba, MD; and Kenichi Tamura, MD

Background and Purpose: We studied the activities of the coagulation-fibrinolysis system in the chronic stage of poststroke patients and the effect of antiplatelet medication on the system.

Methods: We determined fibrinogen, antithrombin III, thrombin-antithrombin III complex, tissue plasminogen activator antigen, plasminogen activator inhibitor-1, plasmin-α, plasmin inhibitor complex, and D-dimer in plasma from 153 poststroke patients in the chronic phase (i.e., 33 patients not receiving antiplatelet medication, 78 patients receiving 200 mg/d ticlopidine, and 42 patients receiving 40 mg/d aspirin), and compared the results in control subjects and among the treatment groups.

Results: The concentrations of fibrinogen, thrombin-antithrombin III complex, antithrombin III, plasmin-α, plasmin inhibitor complex, and tissue plasminogen activator were slightly but significantly increased in all treatment groups compared with control subjects (P<.01) but did not differ among the treatment groups. The plasminogen activator inhibitor-1 levels were significantly elevated in patients not receiving antiplatelet medication compared with control subjects (P<.01), whereas they were in the normal range and significantly lower in patients receiving ticlopidine or aspirin than in patients not receiving antiplatelet medication (P<.01). The plasminogen activator inhibitor-1 levels were significantly lower in patients whose platelet aggregation was inhibited by antiplatelet medication than in patients with uninhibited platelet aggregability (P<.05).

Conclusions: These findings suggest that coagulation-fibrinolysis markers are mildly increased in poststroke patients in the chronic phase and that antiplatelet medication is effective in reducing the elevated plasminogen activator inhibitor-1 levels. (Stroke 1993;24:801–804)

Key Words • aspirin • fibrinolysis • platelet aggregation • ticlopidine • thrombosis

Previous studies have demonstrated that the coagulation-fibrinolysis system was activated in the acute and subacute stages of cerebral thrombosis.1,2 However, the activities of coagulation-fibrinolysis in the chronic stage of poststroke patients are not fully known. A large proportion of these patients receive antiplatelet medication for preventing stroke recurrence. It is well documented that platelets contain coagulant proteins, such as fibrinogen, factors V, VIII, IX, and XIII,3 and also plasminogen activator inhibitor-1,4 and they are closely linked to the coagulation-fibrinolysis system. However, as far as we know, there have been no reports concerning the effects of long-term antiplatelet medication on the coagulation-fibrinolysis system.

In this study, we determined the molecular markers for the coagulation-fibrinolysis system in poststroke patients in the chronic stage, and we compared the results among patients receiving ticlopidine or aspirin and patients not receiving antiplatelet medication.

Subjects and Methods

We studied 153 patients (94 men and 59 women; mean±SD age, 63±11 years) with cerebral thrombosis in the chronic phase (more than 6 months after stroke). Control values were obtained from 69 healthy individuals (28 men and 41 women; mean±SD age, 61±9 years) who participated in the annual mass examination in our districts and had not received any medication. Table 1 compares the backgrounds of patients in the treatment subgroups and control subjects. All poststroke patients had infarcts less than 3 cm (with most less than 1 cm) on the computed tomographic scan taken in the acute phase. We excluded patients with cardiogenic embolism. We also excluded patients who became bedridden after the stroke. Ninety poststroke patients (59%) had hypertension, 40 (26%) had diabetes mellitus, and 29 (19%) smoked. Thirty-three patients (64±13 years) had not received antiplatelet medication, 78 patients (62±10 years) had received 200 mg/d ticlopidine, and 42 patients (64±11 years) had received 40 mg/d aspirin for more than 6 months. We could not randomly assign the patients to the three groups, and many of the patients free from antiplatelet medication had a history of gastrointestinal ulcers. However, there was no significant difference in the backgrounds among the three treatment groups, although the incidence of men, smokers, diabetics, and patients having high hematocrit levels tended to be greater in patients on antiplatelet medication than in patients not receiving antiplatelet medication (Table 1). None of the patients received warfarin. Patients with hypertension and/or diabetes were treated with appropriate medication.

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TABLE 1. Backgrounds of Patients in Treatment Subgroups and Control Subjects

<table>
<thead>
<tr>
<th>No antiplatelet medication (n=33)</th>
<th>Ticlopidine (n=78)</th>
<th>Aspirin (n=42)</th>
<th>Control subjects (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean±SD)</td>
<td>64±13</td>
<td>62±10</td>
<td>64±11</td>
</tr>
<tr>
<td>Male</td>
<td>16 48</td>
<td>26 62</td>
<td>28 41</td>
</tr>
<tr>
<td>Smoking</td>
<td>9 27</td>
<td>14 33</td>
<td>22 32</td>
</tr>
<tr>
<td>Hypertension</td>
<td>22 67</td>
<td>22 58</td>
<td>14 20</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 12</td>
<td>8 19</td>
<td>0</td>
</tr>
<tr>
<td>Total cholesterol &gt;220 mg/dL</td>
<td>8 24</td>
<td>7 17</td>
<td>13 19</td>
</tr>
<tr>
<td>HDL &lt;45 mg/dL</td>
<td>8 24</td>
<td>16 38</td>
<td>15 22</td>
</tr>
<tr>
<td>Triglyceride &gt;150 mg/dL</td>
<td>6 18</td>
<td>6 14</td>
<td>12 17</td>
</tr>
<tr>
<td>Hematocrit &gt;45%</td>
<td>5 15</td>
<td>12 29</td>
<td>14 20</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>0 2</td>
<td>3 0</td>
<td>0</td>
</tr>
</tbody>
</table>

HDL, high-density lipoprotein.

Blood was withdrawn by careful venipuncture, without the use of venous stasis, into a prechilled tube containing 32 mg/mL sodium citrate for fibrinogen, thrombin-antithrombin III complex (TAT), antithrombin III (AT III), tissue plasminogen activator (t-PA) antigen, and plasminogen activator inhibitor-1 (PAI-1). Blood was also collected into a tube containing an anticoagulant solution (500 IU/mL heparin, 10 KIU/mL aprotinin) for plasmin-α2 plasmin inhibitor complex (PIC) and d-dimer. The tubes were centrifuged at 2000g for 15 minutes, and plasma was stored at −20°C until assayed. The fibrinogen concentration was measured by the thrombin time method. TAT in plasma was determined by enzyme-linked immunosorbent assay (ELISA) (Enzygnost TAT kit; Behringwerke, Marburg, Germany). The test system was based on the two-step “sandwich” method, using two different antibodies directed against human thrombin and AT III. Plasma AT III activity was determined by the two-stage method, using the chromogenic substrate H-D-Phe-Pip-Arg-pNA (S-2238) (Testzym AT III auto; Daiichi Pure Chemicals, Tokyo, Japan).6 Plasma concentrations of t-PA antigen were determined by ELISA with Tint Elize t-PA Biopool, Umeå, Sweden.7 PAI-1 in plasma was determined based on the double antibody principle, using two different monoclonal antibodies raised against PAI-1 (PAI-1 ELISA kit, type FI-5; Monozyme, Charlottenlund, Denmark). PIC was assayed by an ELISA one-step sandwich method (EIA-B; Teijin Ltd., Tokyo, Japan), using peroxidase-conjugated monoclonal anti-α2 plasmin inhibitor antibody.8 d-dimer levels were measured by an ELISA6 (Dimer Test EIA; Agen Ltd, Brisbane, Australia).

In 78 patients who underwent platelet aggregation studies, venous blood was put into tubes containing sodium citrate. Aggregation of platelet-rich plasma was measured by percent maximum change in light transmission using a Born aggregometer (Niko Bioscience, Tokyo, Japan); the aggregating reagent used was 10 µM ADP (Sigma Chemical Co, St Louis, Mo).

Intergroup differences of the means were evaluated using two-tailed Student’s t test.

Results

The concentrations of fibrinogen, TAT, and AT III were significantly increased in all three treatment groups compared with the control group and did not differ among the treatment groups (Table 2). There was a significantly positive correlation between the TAT and AT III levels (r=22, P<.01). The concentration of PAI-1 was significantly increased in patients not receiving antiplatelet medication (Table 2). However, the PAI-1 concentration in patients receiving ticlopidine or aspirin was unaltered compared with control subjects and was significantly lower than in patients not receiving antiplatelet medication (P<.01).

The 10 µM ADP-induced platelet aggregability as expressed by the maximum transmission was significantly reduced in patients receiving ticlopidine (55±17%, n=39, P<.01) and in patients receiving aspirin (59±17%, n=27, P<.01) compared with patients not receiving antiplatelet medication (69±11%, n=12). The PAI-1 concentrations were related to the platelet aggregability (Figure). If we place the cutoff level for PAI-1 at the 97.5% confidence limit of the control subjects (50 ng/mL), the incidence of PAI-1 levels higher than this was greater in patients having a platelet aggregability greater than 55% (13/52, 25%) than in patients having a platelet aggregability less than or equal to 55% (1/26, 4%) (χ²=5.3, P<.05).

The t-PA and PIC concentrations were slightly but significantly increased in the treatment groups of the poststroke patients compared with the control group (Table 2); however, the d-dimer levels did not differ significantly in the poststroke patients compared with the control group.

Discussion

Our main findings were (1) that the concentrations of fibrinogen, TAT, AT III, t-PA, and PIC were significantly increased in poststroke patients in the chronic stage; (2) that the PAI-1 levels were elevated in patients not receiving antiplatelet medication but were within the normal range in patients receiving ticlopidine or aspirin; and (3) that the increased PAI-1 levels were significantly associated with an insufficient reduction in
ADP-induced platelet aggregability by the antiplatelet medication.

The observed significant increase in the fibrinogen levels in the chronic period compared with control subjects is consistent with previous findings indicating the sustained elevation of fibrinogen levels of stroke patients and also with the fact that an elevated fibrinogen concentration is a risk factor for stroke. Our control values were obtained from healthy individuals not receiving medication, ie, relatively low-risk subjects. If we include individuals receiving medication for diseases such as hypertension and diabetes, it is possible that the control values for fibrinogen and other coagulation-fibrinolysis markers may be higher.

It has been reported that the TAT levels were increased in myocardial infarction and that the increased TAT levels predicted the risk for reocclusion after successful thrombolysis of coronary arteries. Our results demonstrate that the TAT levels are also increased in poststroke patients in the chronic stage, suggesting a sustained enhancement of the coagulation system. Previous studies have demonstrated a reduction in AT III activity in the acute stage and its recovery to control values in the later stage, suggesting the consumption of active AT III in the acute stage. The present findings indicate that such consumption of AT III does not occur in the chronic stage. The increased TAT levels unassociated with the reduction in the AT III levels may be explained by the fact that the AT III antigen levels in the plasma (approximately 100 to 300 mg/L) are far greater than the TAT levels (1 to 2 µg/L). However, the observed significant positive correlation between the AT III and TAT levels suggests that the increase in TAT levels is associated with higher levels of AT III.

Increased PAI-1 levels have been reported in myocardial infarction and angina pectoris and have been considered to indicate decreased fibrinolytic activity. We also found an increase in PAI-1 levels in poststroke patients in the chronic stage who were not receiving antiplatelet medication. It is unlikely that the increased PAI-1 levels were due to an acute phase reaction, because our patients were studied more than 6 months after the stroke. Yet we do not know the origin of the increased PAI-1. It has been demonstrated that although aspirin inhibited the arachidonate- and collagen-induced PAI-1 release from platelets in vitro, the plasma PAI-1 levels did not change after 1 week of aspirin medication. However, our findings suggest that antiplatelet medication for more than 6 months reduced the plasma PAI-1 levels. Because we used a very low dose (40 mg/d) of aspirin in this study, it is to be determined in further studies whether higher dosages may exert greater effects on the PAI-1 levels. The observed significant correlation between the PAI-1 levels and platelet aggregability suggests that the increased PAI-1 levels may originate largely from platelets. It has been reported that PAI-1, stored in α-granules, is released from activated platelets along with β-thromboglobulin and platelet factor 4. PAI-1 released from platelets is a serine protease inhibitor having the same immunochemical and physicochemical characteristics as that derived from endothelial cells, and it is bound to plasminogen activator to inhibit it. The PAI-1 content in platelets (0.7 ± 0.4 ng/10⁶ platelets; ie, 140 ± 80 ng/mL blood, if the platelet count is 2 × 10⁹/mm³) is comparable to its concentration in the serum (270 ± 68 ng/mL) and far greater than that in plasma (21 ± 7.2 ng/mL). However, the PAI-1 activity rate (PAI-1 activity/PAI-1 antigen) in platelets has been reported as

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**TABLE 2. Coagulation-Fibrinolysis System in Treatment Groups of Poststroke Patients and Control Subjects**

<table>
<thead>
<tr>
<th>Poststroke patients</th>
<th>No antiplatelet medication (n=33)</th>
<th>Ticlopidine (n=78)</th>
<th>Aspirin (n=42)</th>
<th>Control subjects (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>280±79*</td>
<td>272±68*</td>
<td>289±66*</td>
<td>231±43</td>
</tr>
<tr>
<td>TAT (µg/L)</td>
<td>5.7±9.9*</td>
<td>5.2±9.1*</td>
<td>5.3±6.9*</td>
<td>1.5±0.4</td>
</tr>
<tr>
<td>AT III (%)</td>
<td>109±26*</td>
<td>102±17*</td>
<td>104±25*</td>
<td>90±10</td>
</tr>
<tr>
<td>t-PA (ng/mL)</td>
<td>6.2±1.8*</td>
<td>6.5±2.7*</td>
<td>6.5±2.8*</td>
<td>4.2±2.3</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>89±94*</td>
<td>45±63†</td>
<td>45±63†</td>
<td>20±15</td>
</tr>
<tr>
<td>PIC (ng/mL)</td>
<td>1.1±0.7*</td>
<td>1.0±0.5*</td>
<td>1.3±0.7*</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>D-dimer (ng/mL)</td>
<td>136±110</td>
<td>116±76</td>
<td>108±54</td>
<td>100±15</td>
</tr>
</tbody>
</table>

Values are mean±SD. TAT, thrombin-antithrombin III complex; AT III, antithrombin III; t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; PIC, plasmin-α₂ plasmin inhibitor complex.

*P<0.01 vs control.
†P<0.01 vs no antiplatelet medication.

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Plot shows plasma plasminogen activator inhibitor-1 (PAI-1) levels in terms of platelet aggregation induced by 10 µM ADP (percent maximum change in light transmission).
approximately 5% or 20%. It is uncertain whether the increased PAI-1 antigen levels represent functionally active PAI-1 and whether there is actually decreased fibrinolytic activity that might have significance for stroke risk. Correlative assays of fibrinolytic activity and PAI-1 antigen levels and prospective studies with a larger number of patients would answer these questions.

Our present results indicate that t-PA and PIC levels are also slightly elevated in the chronic stage, as has been demonstrated previously in the acute and subacute stages; however, their levels were not affected by antiplatelet medication.

In conclusion, the mean levels of coagulation-fibrinolysis markers are increased in poststroke patients in the chronic stage, and the PAI-1 levels are reduced to normal levels if platelet aggregability is inhibited by antiplatelet medication. The prognostic and therapeutic significance of these findings is open to further studies.

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