Effect of Plasma Fibrinogen Concentration on the Inhibition of Platelet Aggregation After Ticlopidine Compared With Aspirin

Hideo Tohgi, MD; Hiroaki Takahashi, MD; Mitsuru Kashiwaya, MD; Katsumi Watanabe, MD

Background and Purpose Elevated levels of plasma fibrinogen are a risk factor for cerebral infarction. Because fibrinogen plays a central role in platelet aggregation and binding of fibrinogen to platelets is inhibited by ticlopidine, we studied the effect of the plasma fibrinogen concentration on the antiaggregatory action of ticlopidine compared with that of aspirin.

Methods We determined platelet aggregability before and after administration of ticlopidine (200 mg/d) or aspirin (81 mg/d) in 61 stroke patients and correlated the changes with the plasma fibrinogen concentration.

Results In patients receiving ticlopidine, the platelet aggregability induced by 1, 5, and 10 μmol/L adenosine diphosphate significantly decreased compared with aggregability before medication (P<.05), and the reductions had significant negative correlations with the plasma fibrinogen concentration (P<.05). In patients receiving aspirin, the platelet aggregability induced by 2 μg/mL collagen and 5 and 10 μmol/L adenosine diphosphate decreased compared with aggregability before medication (P<.005), but the reductions had no significant correlation with the plasma fibrinogen concentration.

Conclusions The relative antiaggregatory effect of ticlopidine is significantly decreased with higher plasma fibrinogen concentrations. This may explain, at least in part, the individual variation in the response to ticlopidine. (Stroke. 1994;25:2017-2021.)

Key Words • aspirin • cerebrovascular disorders • fibrinogen • platelet aggregation • ticlopidine

Elevated levels of plasma fibrinogen are among the risk factors for ischemic stroke.1 Fibrinogen exerts thrombogenic properties not only by increasing blood viscosity and by participating in the final stages of the blood coagulation process but also by playing a central role in platelet aggregation. In the working model presently acknowledged,2 binding of adenosine diphosphate (ADP) to the 10-kD transmembrane protein of platelets produces a conformational change in the protein and allows the assembly of IIb/IIIa-Ca²⁺, resulting in the exposure of latent fibrinogen binding sites necessary for platelet aggregation. Because the dissociation constant for the fibrinogen binding is far below the normal range of the fibrinogen concentration, aggregation of normal platelets may be virtually unaffected by the fibrinogen concentration within its normal range. Fibrinogen, however, may affect the activity of such antiplatelet agents as ticlopidine, the antiaggregatory action of which is largely derived from the elevation of cyclic adenosine monophosphate (cAMP),3 as well as the inhibition of fibrinogen binding to platelets.4 However, to our knowledge, no data are available concerning the effect of the plasma fibrinogen concentration on the antiaggregatory effect of ticlopidine, which may be a potential source of individual variation in the responses to the medication. We therefore studied the correlations between the fibrinogen concentrations and changes in the platelet aggregability after administration of ticlopidine compared with those after aspirin in poststroke patients.

Subjects and Methods

We studied 61 patients (mean age, 66±10 years; 42 men and 19 women) who were admitted to the hospital because of a minor atherothrombotic stroke. During the acute phase (<7 days after onset), they were treated with 400 to 600 mL/d of 10% glycerol and/or 500 mg/d of dextran 40. Most patients had a lacunar infarction (<1.5 cm). We excluded patients with an embolic stroke based on abrupt onset, atrial fibrillation, and cortical infarction demonstrated on computed tomography or magnetic resonance imaging scans and those who had a large infarction (>3 cm) and had received prolonged treatment (>7 days) for the acute phase. We also excluded patients who had complications from systemic diseases such as pneumonia, urinary tract infection, deep vein thrombosis, or neoplasms. None of the patients had taken nonsteroidal anti-inflammatory medication within 1 month of the study. After the treatments for the acute phase, the patients began a rehabilitation schedule. Two weeks after admission (ie, about 1 week after the end of the acute phase treatment), all of the patients were independent or partly dependent. Thirty patients received ticlopidine (200 mg/d), and 31 patients received aspirin (81 mg/d). A dosage of 200 mg/d ticlopidine is standard for the Japanese population. The study was performed during the patients' stay in the hospital. Attending physicians, nurses, and pharmacists ensured that the patients were taking the prescribed medication. Informed consent was obtained from all patients. Table 1 gives the demographic background of the subjects.

We determined plasma fibrinogen concentration and platelet aggregability before and 2 to 4 weeks after the onset of the antiplatelet medication. Fibrinogen concentration was measured by the thrombin time method. For platelet aggregation...
TABLE 1. Demographic Backgrounds of Subjects by Treatment Group

<table>
<thead>
<tr>
<th></th>
<th>Ticlopidine (n=30)</th>
<th>Aspirin (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y*</td>
<td>65±9</td>
<td>67±11</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>23 (77)</td>
<td>19 (61)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>22 (73)</td>
<td>21 (68)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>14 (47)</td>
<td>10 (32)</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>9 (31)</td>
<td>11 (36)</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>15 (50)</td>
<td>10 (32)</td>
</tr>
</tbody>
</table>

*Mean±SD.

studies, venous blood was taken by venipuncture and put into tubes containing sodium citrate. Aggregation in both whole blood and platelet-rich plasma (PRP) was studied within 30 minutes after venipuncture. In all samples, red blood cell counts (3.5 to =5.0x10^6 cells/μL), white blood cell counts (4 to =8x10^3 cells/μL), and platelet counts (1.5 to =3.5x10^4 cells/μL) were within the normal ranges. Whole-blood platelet aggregation was estimated using a Chrono-Log model 540 whole-blood aggregometer (Coulter Electronics Ltd); aggregating reagents were 2 μg/mL collagen and 10 μmol/L ADP.

Rate of aggregation was assessed by measuring the change in impedance (in ohms) 6 minutes after adding the reagents. A linear relation between changes in impedance at 6 minutes and the maximum changes had been confirmed. The PRP aggregation was measured by percent maximum change in light transmission using a Born aggregometer (Niko Bioscience). The aggregating reagents used were 2 μg/mL collagen (Chrono-Log) and 1, 5, and 10 μmol/L ADP (Sigma Chemical Co).

Comparisons of data between before and after medication were made with paired t tests (two-tailed). We also evaluated the correlations between platelet aggregability and fibrinogen concentrations both before and after medication. In addition, correlations were determined between the before-after differences in platelet aggregability and in fibrinogen concentrations after medication. All data were tested for normality. A few data that were not normally distributed were evaluated with Spearman's rank correlation coefficients. Otherwise, Pearson product-moment correlation coefficients (with their 95% confidence limits calculated using Fisher's r/z transformation) were used.

Results

There was no significant difference in the demographic backgrounds of the ticlopidine-treated and aspirin-treated patients (Table 1) or in the fibrinogen concentrations and platelet aggregability (Table 2) before and after medication.
Table 3. Correlation Coefficients Between the Differences in Platelet Aggregability Before and After Antithrombotic Therapy (Before Minus After) and Plasma Fibrinogen Concentration After Therapy

<table>
<thead>
<tr>
<th></th>
<th>Correlation Coefficients (95% Confidence Limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ticlopidine (200 mg/d; n=30)</strong></td>
<td></td>
</tr>
<tr>
<td>Impedance method, Ω</td>
<td></td>
</tr>
<tr>
<td>Collagen (2 μg/mL)</td>
<td>0.18 (−0.19, 0.50)</td>
</tr>
<tr>
<td>ADP (10 μmol/L)</td>
<td>−0.39 (−0.04, −0.66)*</td>
</tr>
<tr>
<td>Light transmission method, %</td>
<td></td>
</tr>
<tr>
<td>Collagen (2 μg/mL)</td>
<td>0.27 (−0.10, 0.57)</td>
</tr>
<tr>
<td>ADP (1 μmol/L)</td>
<td>−0.41 (−0.06, −0.67)*</td>
</tr>
<tr>
<td>ADP (5 μmol/L)</td>
<td>−0.45 (−0.11, −0.70)*</td>
</tr>
<tr>
<td>ADP (10 μmol/L)</td>
<td>−0.46 (−0.14, −0.70)*</td>
</tr>
</tbody>
</table>

**Aspirin (81 mg/d; n=31)**

| Impedance method, Ω             |                                                  |
| Collagen (2 μg/mL)              | 0.24 (−0.13, 0.55)                              |
| ADP (10 μmol/L)                 | −0.01 (−0.36, 0.35)                             |
| Light transmission method, %    |                                                  |
| Collagen (2 μg/mL)              | 0.28 (−0.08, 0.58)                              |
| ADP (1 μmol/L)                  | −0.03 (−0.36, 0.33)                             |
| ADP (5 μmol/L)                  | 0.05 (−0.31, 0.40)                              |
| ADP (10 μmol/L)                 | 0.02 (−0.34, 0.37)                              |

ADP indicates adenosine diphosphate. *P < 0.05.

Discussion

Our findings confirm that ticlopidine more profoundly inhibits ADP-induced than collagen-induced aggregation and that aspirin inhibits the secondary phase of platelet aggregation. The fibrinogen concentration did not significantly change with either ticlopidine or aspirin. The latter finding is consistent with some previous reports but not with other studies demonstrating a significant decrease in the fibrinogen concentration after ticlopidine administration. This discrepancy may be in part due to the difference in subjects and in the doses of ticlopidine. An important finding is the significant negative correlations of post-treatment fibrinogen levels with the relative antiaggregatory effects of ticlopidine demonstrated by the before-after difference in ADP-induced aggregation measured by impedance and light transmission method.

ADP concentrations and platelet counts (before-after differences) in both patient groups (Table 2). In patients receiving ticlopidine, platelet aggregability induced by 1, 5, and 10 μmol/L ADP and determined by the light transmission method was significantly decreased compared with pretreatment values (P < 0.05) (Table 2). In patients receiving aspirin, platelet aggregation induced by collagen (with both impedance and light transmission methods) and by 5 and 10 μmol/L ADP (with the light transmission method) was significantly decreased compared with pretreatment values (P < 0.05) (Table 2). Fibrinogen concentrations had no significant correlations with absolute platelet aggregability induced by different stimuli and determined by different methods before and after medication in both the ticlopidine- and aspirin-treated groups (data not shown). However, among ticlopidine-treated patients, the fibrinogen concentration after medication had significant negative correlations with the differences between before and after medication (value before medication minus value after medication) in the aggregability induced by 10 μmol/L ADP (both impedance and light transmission methods) and 1 and 5 μmol/L ADP (light transmission method) (P < 0.05) (Table 3; Figure, A through D). In aspirin-treated patients, no such correlations were obtained (Table 3).
ods; there were no such correlations for aspirin. These results suggest that higher fibrinogen levels may play a role in decreasing the relative antiplatelet effects of ticlopidine. The before-after changes in aggregability in relation to fibrinogen levels in ticlopidine-treated patients were remarkable; an increase in the fibrinogen levels by 100 mg/dL was associated with a 5-8-fold decrease in the before-after difference in aggregation determined by the impedance method and with an ≈10% decrease in the difference by light transmission (Figure). Although the degree of antiplatelet activity necessary for reducing stroke risk has not been established, it is possible that the relative reduction in the antiplatelet effect of ticlopidine associated with higher fibrinogen levels may have clinical significance in some patients. It is also important to note, however, that our results were based on a dosage of 200 mg/d ticlopidine, which is less than half the daily dose used in North America and Europe. In addition, our subjects were Japanese who primarily had a lacunar stroke. Whether our hypotheses hold with higher doses, a different patient population, or types of stroke requires further studies.

The potential effect of fibrinogen on platelet aggregation after administration of ticlopidine may be explained by the current model of fibrinogen binding to platelets. Although some earlier studies reported a single class of binding sites,15,16 later studies demonstrated two sets of binding sites (high and low affinity).17,18 which were explained by a negatively cooperative interaction between fibrinogen receptors.18 In one such study, Di Minno et al19 reported a high-affinity site binding 1000 to 1600 fibrinogen molecules per platelet with a dissociation constant (Kd) of 0.029 to 0.045 μmol/L and a low-affinity site binding 46 000 to 76 000 fibrinogen molecules per platelet with a Kd of 1.2 to 2.0 μmol/L (41 to 68 mg/dL). Other researchers reported similar values for Kd (0.25 to 5.6 μmol/L, 10 to 190 mg/dL). Therefore, the changes in the fibrinogen concentration within the normal range (200 to 400 mg/dL) do not substantially affect platelet aggregability. Ticlopidine did not influence the apparent affinity for fibrinogen of the high-affinity sites, but it significantly decreased the affinity of the low-affinity sites associated with an 8- to 12-fold increase of Kd (410 to 680 mg/dL),19 which is above the normal range of the fibrinogen concentration. Therefore, after ticlopidine administration, the fibrinogen binding increases almost linearly with the increasing concentration of fibrinogen and thus may augment platelet aggregation.

The correlation between platelet aggregability after ticlopidine administration and the fibrinogen concentration did not reach a significant level. Therefore, the effects of plasma fibrinogen levels on ticlopidine’s antiplatelet action were only relative. This is probably because of individual variation within the normal range in other parameters influencing platelet aggregability, such as platelet count and calcium concentration. In the whole-blood impedance method, variations within the normal range in red blood cell and white blood cell counts may also obscure the changes in platelet aggregability. Moreover, it is probable that the degrees of involvement of cAMP synthesis and its inhibition by ticlopidine differ among individuals. The comparisons in aggregability before and after medication for individual patients may have minimized the influences of these variables. High levels of fibrinogen, however, appear to be only one of the reasons for a missing reduction of platelet aggregation under ticlopidine, for the correlation coefficients (r = -0.39 to -0.46) (Table 3) suggest that fibrinogen levels are responsible for only 15% to 21% (r² = 0.15 to 0.21) of the variance in the difference in platelet aggregation before and after ticlopidine. Although we selected patients who had had a minor stroke to avoid the influences of treatments for the acute phase, our hypothesis would be better tested in healthy volunteers who are free of such potential confounders.

The lack of a correlation between the fibrinogen concentration and the antiaggregatory effect of aspirin appears to be consistent with a lack of an effect of aspirin on the affinity of platelets for fibrinogen binding.

In conclusion, because the fibrinogen concentration appears to account, in part, for individual variations in response to ticlopidine, it may be one of the variables that must be considered in evaluating the effects of antiplatelet agents.

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