Compliance With Antiplatelet Therapy in Patients With Ischemic Cerebrovascular Disease Assessment by Platelet Aggregation Testing

Tadatoshi Komiya, MD; Manabu Kudo, MD; Takao Urabe, MD; Yoshikuni Mizuno, MD

**Background and Purpose** Antiplatelet therapy is currently one of the methods for preventing transient ischemic attacks and cerebral thrombosis. Because antiplatelet agents are generally administered on a long-term basis, patient compliance is an important factor. The purpose of this study was to determine the compliance of patients during antiplatelet therapy by testing platelet aggregation.

**Methods** To establish the conditions for measuring platelet aggregation, the platelet aggregation test was performed in patients taking 81 mg/d aspirin or 200 mg/d ticlopidine at the following final concentrations of aggregation-inducing agents: 0.5, 1, 2, and 4 μmol/L ADP and 0.5 and 2 μg/mL collagen.

The optimum measurement conditions for assessing patient compliance were determined. Under the conditions determined in the first study, platelet aggregation was assessed, and the effects of treatment were studied in 159 outpatients and 79 inpatients undergoing antiplatelet therapy. If the antiplatelet effect was insufficient, compliance was checked by interview.

**Results** The agents used and the final concentrations found to be optimum for assessing platelet aggregation were 2 μg/mL collagen for patients taking aspirin 81 mg/day and 2 μmol/L ADP for patients taking ticlopidine 200 mg/d. In 17 (10%) of the 159 outpatients, platelet aggregation was not adequately reduced because of noncompliance with their antiplatelet therapy.

**Conclusions** This study indicated that monitoring of compliance is important for outpatients on antiplatelet therapy. It is best if platelet aggregation can be checked, but when this is impossible it is necessary to assess compliance periodically and provide patient guidance. (Stroke. 1994;25:2337-2342.)

**Key Words** • antiplatelet agents • cerebral infarction • patient compliance • platelet aggregation

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The efficiency of aspirin for the prevention and treatment of transient ischemic attacks (TIAs) has been proven, and its efficacy in the secondary prevention of cerebral thrombosis has also been confirmed.1-3 The most common dosage used is 300 mg/d or more,4 but it has also been reported that aspirin is effective at lower dosages (30 to 300 mg/d).5-8 Ticlopidine has been proven to have approximately a 20% greater preventive effect than aspirin.9-10 Although both of these drugs have a significant antiplatelet effect, it has been difficult to define standard dosages for their administration; such factors as mean body weight and pharmacological effect need to be studied in patients from various countries. In Japan, antiplatelet therapy is widely used for the prevention and treatment of TIA and cerebral thrombosis, with the usual dosages being 81 to 300 mg/d aspirin or 200 mg/d ticlopidine.

Antiplatelet therapy is given for long periods ranging from a few years to 30 to 40 years, suggesting that control of patient compliance by monitoring the effect of the antiplatelet drug may be important. There are several studies of monitoring of the effect of chronic antiplatelet therapy.11-14 Determinations of blood levels of thromboxane B2 and assessment of platelet aggregation are important for monitoring the effects of antiplatelet agents. Although measurement of the blood level of drugs such as aspirin is possible, it is not done routinely because of the rapid metabolism of these agents.15 The pharmacological effects of ticlopidine result from the actions of its metabolite, but the blood level cannot be measured because this metabolite has still not been identified.16 Accurate determination of thromboxane B2 levels is possible, but this parameter cannot be used to distinguish the pharmacological actions of aspirin and ticlopidine because it only shows the total effect on platelet aggregation.

In this study, platelet aggregation tests were performed on patients taking the standard Japanese dosages of aspirin or ticlopidine. The most useful agents and the optimum concentrations for monitoring platelet function were decided, and the working diagnostic thresholds for compliance of aspirin and ticlopidine were determined. Then platelet aggregation was assessed under the conditions determined in the first study, and the compliance of outpatients and inpatients on antiplatelet therapy was investigated.

**Subjects and Methods**

**Establishment of the Appropriate Conditions for the Platelet Aggregation Test**

Sixty-one patients (10 with TIA and 51 with previous cerebral thrombosis) were selected as subjects to determine the conditions for assessing platelet aggregation. This total comprised 43 men (mean age, 66±9 years) and 18 women (mean age, 69±6 years). Twenty-eight of these patients took aspirin and 33 took ticlopidine. After receiving the patients' consent, administration of the antiplatelet agent was withheld for more than 10 days before study.
was determined.

The platelet aggregating agents used and their final concentrations for evaluating the effects of the antiplatelet agents were determined, and the working diagnostic threshold of each drug was investigated.

The paired $t$ test was used for statistical analysis.

### Compliance Study

Platelet aggregation was tested in patients undergoing antiplatelet therapy using the measurement conditions determined in the first study. The timing of the test was random. In principle, the test was performed once on each patient, but it was repeated when abnormal values outside the working diagnostic threshold were obtained. Patients were interviewed to determine whether their compliance was satisfactory, and the cause of any abnormal results was clarified. The contents of the interview were as follows: "The effects of the antiplatelet agent were examined but were not adequate. Did you take the drug as directed?" The patient answered yes or no. If there was noncompliance, the following two steps were taken on the basis of the protocol. (1) Remaining pills were counted, and the period during which the patients did not take pills was checked. (2) If readministration was possible, the patients were given the drug again by the investigators, and platelet aggregation was measured again. The dosage of each antiplatelet agent was 81 mg/d for aspirin and 200 mg/d for ticlopidine.

### Results

Platelet Aggregation Test

The Table summarizes the results obtained. Before drug administration, the maximum aggregation rate increased with increasing concentrations of ADP and collagen in each group of subjects. The mean maximum aggregation rate at 0.5 $\mu$g/mL collagen in the ticlopidine group was higher than in the aspirin group; it appears that more patients who were apt to show aggregation by collagen at a low concentration were included in the ticlopidine group than in the aspirin group.

When the measurements were repeated after drug administration, there was a greater inhibition rate of

<table>
<thead>
<tr>
<th>Group</th>
<th>ADP, $\mu$mol/L</th>
<th>Collagen, $\mu$g/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Aspirin (n=28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum aggregation rate, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before administration</td>
<td>28±13</td>
<td>46±17</td>
</tr>
<tr>
<td>Aspirin 81 mg/d</td>
<td>24±8</td>
<td>38±11</td>
</tr>
<tr>
<td>Inhibition rate, %</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Ticlopidine (n=33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum aggregation rate, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before administration</td>
<td>30±12</td>
<td>52±20</td>
</tr>
<tr>
<td>Ticlopidine 200 mg/d</td>
<td>15±5*</td>
<td>26±7*</td>
</tr>
<tr>
<td>Inhibition rate, %</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Values are mean±SD. *$P<.01$ compared with pretreatment values.
collagen-induced aggregation than ADP-induced aggregation in the aspirin group (Table). As shown in Fig 1, many patients already had a low maximum aggregation rate even before the administration of aspirin at 0.5 μg/mL collagen. At 2 μg/mL collagen, the maximum aggregation rate showed a marked difference before and after administration, and this concentration appeared to be useful for the evaluation of drug efficacy.

In the ticlopidine group, the inhibition rate of ADP at concentrations of 0.5, 1, 2, and 4 μmol/L was highest at 2 μmol/L, but the mean values were very similar for 0.5, 1, and 2 μmol/L (Table). However, at 0.5, 1, and 4 μmol/L, it was difficult to evaluate the effects because there was overlap of the pretherapy and posttherapy values (Fig 2). On the other hand, with the maximum aggregation rate at 2 μmol/L ADP, there was less overlap of the values obtained before and after drug administration, and this test was more useful for the evaluation of treatment efficacy. The inhibition rate of the maximum aggregation of 0.5 μg/mL collagen was slightly greater than the inhibition rate of ADP (Table), but the aggregation rate was already low before drug administration in many cases, making evaluation difficult.

These results indicated that determination of the maximum aggregation rate using 2 μg/mL collagen provided the best evaluation of the effect of aspirin. For evaluation of the effect of ticlopidine, however, the maximum aggregation rate achieved with 2 μmol/L ADP was the most appropriate.

Next, the working diagnostic threshold for compliance of the two antiplatelet agents was determined using the assays mentioned. In the aspirin group, the maximum aggregation rate with 2 μg/mL collagen was reduced to under 60% (Fig 1, right). The maximum aggregation rate with 2 μmol/L ADP was also reduced to less than 60% in the ticlopidine group (Fig 2, lower left). These results indicated that the working diagnostic threshold was less than 60% of the maximum aggregation induced by 2 μg/mL collagen when aspirin was administered at 81 mg/d and less than 60% of the maximum aggregation induced by 2 μmol/L ADP when ticlopidine was given at 200 mg/d.

**Compliance**

A total of 238 subjects was selected for the compliance control study. Of this total, 128 patients were receiving aspirin (87 outpatients and 41 inpatients), and 110 patients were receiving ticlopidine (72 outpatients and 38 inpatients). The period from the start of drug administration until performance of the platelet aggregation test was 1 to 7 years (mean, 3.5 years) for the patients taking aspirin and 1 to 5 years (mean, 3.1 years) for those taking ticlopidine. Two inpatients were given ticlopidine by a pernasal intragastric tube, and all the other patients took their drugs orally.

When the platelet aggregation test was performed randomly under the conditions determined in the first study, patients with limited reduction of aggregation outside the working diagnostic threshold were observed (Fig 3). The patients with maximum aggregation rates exceeding 60% included 13 (15%) of the 87 patients taking aspirin, 6 (8%) of the 72 patients taking ticlopidine, and 2 (5%) of the 38 inpatients taking ticlopidine. These 21 patients showed secondary aggre-
activation with ADP. Interview of 19 of the outpatients with no aggregation reduction indicated that 17 had not taken the prescribed drug (aspirin, 11 patients; ticlopidine, 6 patients). For these 17 noncompliance patients, the time during which they did not take drugs varied between 1 and 6 months (average, 2.3 months). The remaining 2 patients (both taking aspirin) could not be interviewed because they did not return to the hospital. Of the 17 patients showing noncompliance, 10 consented to take the same drug as directed, and the maximum aggregation rate was found to be below the working diagnostic threshold. These 10 patients forgot to take the drug, and the noncompliance of the other 7 patients was considered to be caused by an adverse reaction to the antiplatelet agent (1 with epistaxis and 5 with abdominal discomfort in the aspirin group, 1 with diarrhea in the ticlopidine group); they were not asked to take the same drug again. Aggregation rates of two of the inpatients taking ticlopidine were also outside the working diagnostic threshold. Both patients were given the drug through a pernasal intragastric tube, but why its effect was not adequate remained unclear. These results indicated that the drug effect was insufficient in 21 (8%) of the 238 patients on antiplatelet therapy and that 17 (7%) were not taking the prescribed drug. All of these patients with poor compliance were outpatients, and they comprised 10% of the 159 outpatients investigated.

**Discussion**

**Establishment of the Appropriate Conditions for the Platelet Aggregation Test**

It has been noted that platelet activation occurs in patients with TLAs and chronic cerebral thrombosis, and this provides the theoretical basis for antiplatelet therapy. However, it has been reported that this functional activation is not directly connected with the occurrence of thrombosis; there are actually cases where activated platelets are consumed by cerebral thrombosis and the level of aggregability is reduced, and there is no consistent correlation between bleeding time or thromboxane B2 levels and platelet aggregability. In addition, thromboxane B2 determination is not a direct measurement of platelet aggregation. The changes in platelet aggregability may also depend on the individual or the methods used. Therefore, considerable caution is required in the interpretation of tests of platelet function. However, maximum platelet aggregability in response to ADP and collagen is a practical test. It appears that the measurement of platelet function in vitro allows one to monitor patients for presumptive evidence of desired aspirin or ticlopidine effect.

Determination of platelet aggregation by aggregometer involves adding an aggregating agent to plasma-containing platelets (PRP and PPP) and observing the changes in light absorbance. However, it is necessary to establish the nature and concentration of the aggregating agent on the basis of the type and dose of the antiplatelet agent being administered. The aggregating agents used in this study included ADP and collagen. ADP acts on platelets via the ADP receptor and collagen acts via the combined glycoprotein (GP) Ia/IIa receptor. When platelet aggregability is increased or high concentrations of ADP or collagen are present, a large amount of ADP from the dense granules, or fibrinogen from the alpha granules, of the platelets will be released. Then binding of fibrinogen to the GPIIb/IIIa receptor on the platelet membrane surface will be promoted, and irreversible aggregation (secondary aggregation) will occur. When platelet aggregation is reduced by the administration of antiplatelet agents or because of a low concentration of ADP, there is no release from the granules, and the aggregated platelets become separated after weak aggregation (primary aggregation). Because collagen promotes the release of ADP from the dense granules and thus causes aggregation, there is a time lag until aggregation occurs. However, in some cases (especially in patients taking antiplatelet drugs) of ADP aggregation, it is difficult to differentiate primary and secondary aggregation. In the case of collagen aggregation, primary and secondary aggregation cannot be differentiated, unlike with ADP aggregation. For these reasons, in the present study the evaluation was determined by the maximum aggregation, ie, the maximum level of aggregation. Physiologically, collagen is also important in platelet adhesion, the stage before aggregation. Aggregation is induced by the exposure of the collagen lying beneath vascular endothelial cells, and collagen is a factor in arterial thrombosis and arteriosclerosis.

The present study showed that using collagen in the assessment of platelet aggregation was useful in evaluating the effects of aspirin, while the platelet aggregation test using ADP was useful for evaluating the effects of ticlopidine. Because aspirin inhibits platelet aggregation by arachidonic acid and epinephrine, the use of these substances that promote aggregation appears to be appropriate. However, arachidonic acid is only marginally soluble and is probably not suitable for routine examinations.

The working diagnostic threshold for aspirin was defined as a reduction of the maximum aggregation rate to less than 60% at a final collagen concentration of 2 μg/mL. The working diagnostic threshold for
ticolipidine was a reduction of the maximum aggregation rate to under 60% at a final ADP concentration of 2 \( \mu \text{mol/L} \). Patients with a low aggregation rate before treatment present a problem with regard to platelet aggregation testing for the assessment of compliance. Platelet aggregation is inhibited when analogues (aspirin, indomethacin, etc) are administered. Therefore, when platelet aggregation is assessed before administration of the antiplatelet agent, it is necessary to check whether other drugs that affect platelet function have been administered.

It is clear from the present study that platelet aggregation can be inhibited by a small dose of aspirin (81 mg/d) or by 200 mg/d ticlopidine. These are the standard dosages used in Japan, but no large-scale clinical study on their preventive effect has been performed. These drugs have different mechanisms of platelet aggregation inhibition. Aspirin irreversibly inhibits platelet cyclooxygenase activity and thus inhibits the synthesis of thromboxane \( A_2 \) from arachidonic acid, so aggregation due to thromboxane \( A_2 \) does not occur. Recent studies on ticlopidine have shown that a metabolite produced in the liver blocks ADP-receptor function. As a result, the binding of GPIIb/IIIa and fibrinogen is inhibited and platelet aggregation is inhibited.\(^{16,26}\) The dosages used in many studies on the preventive effect of these drugs range from 300 to 1300 mg/d for aspirin\(^{9}\), and 500 mg/d for ticlopidine.\(^{9,10}\) The present study showed that platelet aggregation was inhibited by lower doses of these drugs and that the pattern of aggregation differed depending on the drug. The platelet aggregation tests using ADP and collagen as determined in this study probably also can be applied to antiplatelet therapy at higher doses for the assessment of compliance. However, it has been reported that platelet aggregation is not sufficiently inhibited by low doses of aspirin.\(^{11,27,28}\) Accordingly, the present study might suggest that Japanese patients are very susceptible to antiplatelet agents.

**Patient Compliance**

Testing of platelet aggregation under the conditions determined in this study showed that 17 (10%) of 159 outpatients were outside the working diagnostic threshold because of noncompliance. Eleven (13%) of the 87 patients taking aspirin showed no reduction of platelet aggregation because of poor compliance, and the reason was not clear in another 2 (2%) cases. In these 2 patients, it was possible that the cause was either noncompliance or an insufficient dose of aspirin. If the dose is too low, it is necessary to increase it depending on the individual state.\(^{11,14}\) No reduction of platelet aggregation occurred because of noncompliance in 6 (8%) of the 72 outpatients taking ticlopidine. Among the inpatients, the aggregation reduction was insufficient when administration was through a pernasal intra-gastric tube. Although the reason for this was not clear, it is possible that delivery of the drug was not effective with this method of administration.

In this compliance study, we cannot completely deny that some patients who show platelet aggregability below the working diagnostic threshold may be in noncompliance because platelet aggregation may depend on the individual.\(^{11,14}\) Therefore, comparing aggregation levels in an individual before and after therapy might provide better monitoring of compliance. Furthermore, when platelet aggregation is not inhibited sufficiently, we should consider the possibility of not only noncompliance but also aspirin resistance\(^{13}\) and incorrect dosage. The percentage of patients with poor compliance is likely to differ at each institution, but measurement of platelet aggregation should be performed once or twice a year during antiplatelet therapy to confirm relative efficacy or compliance. When platelet aggregation cannot be measured, noncompliance generally can be prevented by making efforts to check compliance periodically, explaining the adverse reactions of the drugs, and obtaining the cooperation of family members when the patient suffers from dementia. There is still no clear answer as to how long antiplatelet therapy should be continued. However, administration for rather long periods is necessary, and good compliance may be important for obtaining a correct understanding of the preventive effect of such therapy.

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