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.

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

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, 68±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.

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Platelet Aggregation Before and After Aspirin or Ticlopidine Administration

<table>
<thead>
<tr>
<th>Group</th>
<th>ADP, μmol/L</th>
<th>Collagen, μg/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>
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<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.

Compliance Control Study

A total of 238 subjects (46 with TIA and 192 with previous cerebral infarction) were selected at random for the performance control study. One hundred fifty-nine of these patients were outpatients and 79 were inpatients. There were 159 men (mean age, 69±9 years) and 79 women (mean age, 69±8 years). They included 128 patients receiving aspirin (87 outpatients and 41 inpatients) and 110 patients receiving ticlopidine (72 outpatients and 38 inpatients).

Establishment of the Appropriate Conditions for the Platelet Aggregation Test

It was confirmed by direct interview that all of the subjects were taking their drugs as directed. The aspirin and ticlopidine were supplied by the investigators. There were 28 patients taking 81 mg aspirin (once in the morning) and 33 patients taking 200 mg of ticlopidine (100 mg morning and evening) daily. Blood samples were taken before and 4 weeks after drug administration was resumed. Whole blood (4.5 mL) was taken from the cubital vein at approximately 11 AM and was sampled with the two-syringe technique after the addition of 0.5 mL of 3.8% citric acid. The sample was kept at room temperature, and measurements were made within 3 hours after blood sampling. No drugs such as anticoagulants or analgesics were administered for 10 days before blood sampling. When chyluria or hemolysis was found, the blood samples were discarded, and the test was performed again.

Platelet aggregation was measured by aggregometer at a wavelength of 660 nm. Platelet-rich plasma (PRP) was prepared by centrifuging the resultant supernatant at 1681g for 10 minutes at room temperature. PRP with a platelet count of 25 to 35×10^9/μL was also prepared from the initial PPP samples. The platelet aggregating agents used and their final concentrations were ADP at 0.5, 1, and 2 μmol/L (Sigma Chemical Co) and collagen at 0.5 and 2 μg/mL (Kyoto Daicichi Co, Ltd). Measurements were performed by adding 22 μL of one of the aggregating agents to 200 μL of PRP in a thermostat-controlled tank at 37°C with agitation at 1000 rpm. Aggregation curves were analyzed automatically using a microcomputer, and the maximum aggregation rate was determined.

The maximum aggregation rate was defined as the maximum percent change in light transmittance with PRP after addition of the aggregating agent, when the transmittance with PRP before addition of the aggregating agent was defined as 0% and that with PPP as 100%. The inhibition rate was obtained as follows both before and after drug administration: inhibition rate = (mean maximum aggregation rate before administration − mean maximum aggregation rate after administration)/mean maximum aggregation rate before administration)×100%. The optimum aggregating agents and 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 μg/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 the platelet 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.

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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 \( \mu \)g/mL collagen. At 2 \( \mu \)g/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 \( \mu \)mol/L was highest at 2 \( \mu \)mol/L, but the mean values were very similar for 0.5, 1, and 2 \( \mu \)mol/L (Table). However, at 0.5, 1, and 4 \( \mu \)mol/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 \( \mu \)mol/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 \( \mu \)g/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 \( \mu \)g/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 \( \mu \)mol/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 \( \mu \)g/mL collagen was reduced to under 60% (Fig 1, right). The maximum aggregation rate with 2 \( \mu \)mol/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 \( \mu \)g/mL collagen when aspirin was administered at 81 mg/d and less than 60% of the maximum aggregation induced by 2 \( \mu \)mol/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 outpatients taking aspirin, 6 (8%) of the 72 outpatients taking ticlopidine, and 2 (5%) of the 38 inpatients taking ticlopidine. These 21 patients showed secondary aggrega-

![Graph showing maximum aggregation rate for each collagen concentration in patients taking 81 mg/d aspirin (ASA). The solid line indicates the pretreatment values and the dashed line the posttreatment values. The y axis (frequency) is divided into two-point sectors, and the number of patients with platelet aggregation rates in each of these sectors is shown. These values are connected by solid or dashed lines. Therefore, a flat linear line is obtained when the frequencies are the same.](http://stroke.ahajournals.org/)

![Graph showing maximum aggregation rate for each ADP concentration in patients taking 200 mg/d of ticlopidine (TIC). The solid line indicates the pretreatment values and the dashed line the posttreatment values. Refer to Fig 1 legend for a complete explanation.](http://stroke.ahajournals.org/)
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
ticlopidine was a reduction of the maximum aggregation rate to under 60% at a final ADP concentration of 2 μmol/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 analgesics (aspirin, indomethacin, etc) are administered. Therefore, when platelet aggregation is assessed before administration of the antplatelet 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 A2 from arachidonic acid, so aggregation due to thromboxane A2 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 GPIIIa/IIIa and fibrinogen is inhibited and platelet aggregation is inhibited. When administration was through a pernasal intragastric tube. Although the reason for this was not clear, it is possible that delivery of the drug was not effective due to the pattern of aggregation differing depending on the drug. The platelet aggregation tests using ADP and collagen as determined in this study probably also can be applied to antplatelet 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. Accordingly, the present study might suggest that Japanese patients are very susceptible to antplatelet 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 outpatients taking aspirin showed no reduction of platelet aggregation due to thromboxane A2 does not occur.

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. 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 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 antplatelet 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 antplatelet 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.

Acknowledgments

The authors wish to thank Junko Otake and Shizuko Ono for their cooperation in the platelet aggregation study. They also wish to thank Professor Kyoshi Saito and Assistant Professors Masanori Ito and Hajime Arai for providing their facilities.

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Compliance with antiplatelet therapy in patients with ischemic cerebrovascular disease.  
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*Stroke*. 1994;25:2337-2342  
doi: 10.1161/01.STR.25.12.2337

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231  
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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