Platelet Function Tests in Thrombotic Cerebrovascular Disorders

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SUMMARY  A variety of platelet function tests were performed in patients with four forms of obstructive cerebrovascular disease (CVD); transient ischemic attacks (TIA), reversible ischemic neurological deficit (RIND), cerebral infarct, and cerebral embolism of cardiac source in rheumatic valvular heart disease (RVHD). Platelet studies included platelet aggregation induced by ADP and ristocetin, spontaneous platelet aggregation, von Willebrand factor (VIII:vWF), platelet aggregation enhancing factor (PAEF), and percentage of large platelets (megathrombocytes). Serial testing was carried out in acute stroke patients. The effect of aspirin therapy was also evaluated. A clear difference in results was observed between patients with cardioembolic and those with other forms of CVD. In patients with TIA, RIND, and cerebral infarct, platelet aggregation, both induced and spontaneous, was enhanced along with elevation of plasma VIII:vWF and PAEF, and increased percentage of megathrombocytes. In patients with cardioembolic embolism, however, these studies were negative except for percent megathrombocytes. This value was increased in the embolic patients with RVHD in comparison with non-embolic patients with RVHD. Increase in platelet aggregation to ADP and percent megathrombocytes developed slowly over a week following stroke. Induced and spontaneous platelet aggregation, and percent megathrombocytes could be normalized with 600 mg aspirin p.o. These studies suggest that a systemic increase of hyperaggregable platelets and of plasma activators of platelet function exists in thrombotic CVD and may be related to its pathogenesis, while local hemodynamic factors may be more important in the thrombogenesis of cardioembolic embolism.

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AN EARLY ARTERIAL THROMBUS consists largely of platelets. This suggests that platelets play an important role in arterial thrombogenesis in vivo. Platelet aggregation is reported increased in patients with TIA or cerebral infarct. 1-7 The purpose of this study was 1) to confirm the participation of platelets in thrombogenesis in thrombotic CVD by performing tests of different aspects of platelet reactions, 2) to study the time course of platelet function in acute stroke patients by frequent serial testing, 3) to examine the relationship between platelet function and various types of ischemic CVD as well as clinical severity, and 4) to estimate the efficacy of an antplatelet agent, aspirin in modifying various platelet function tests.

Materials and Methods

Patients and Controls Studied

Tests were performed in patients with TIA, RIND, cerebral infarct, and cerebral embolism originated from RVHD. All patients were evaluated by the staff of the Neurological Institute of Tokyo Women's Medi-
Platelet Aggregation Enhancing Factor (PAEF)

This activity was determined according to the method of Kwaan et al.\textsuperscript{12, 13} using citrated platelet poor plasma (PPP). Twenty five \(\mu\)l of test plasma was mixed with 200 \(\mu\)l of normal PRP obtained from normal healthy adult volunteers. The mixture was studied for aggregation by the addition of 25 \(\mu\)l of 10\(^{-5}\) M ADP. The activity of PAEF was expressed as the increase in percentage of optical transmission at four minutes after the addition of ADP. PAEF activity was measured in 32 patients (27 males, 5 females) with cerebral infarct (mean age of 60 years; range 27–77 years), 26 patients (18 males, 8 females) with TIA/RIND (mean age of 60 years; range 27–77 years), 7 patients (5 males, 2 females) with RVHD and cerebral embolism (mean age of 43 years; range 28–59 years) and the same number of age- and sex-matched controls for each group.

Spontaneous Platelet Aggregation (SPA)

SPA was determined by using an aggregometer (NKK Hematrace I) and stirring the fresh citrated PRP sample by a metal bar (length 5 mm; diameter 1 mm) without the addition of any inducer.\textsuperscript{14, 15} The extent of SPA was expressed as the percent maximum change of light transmission on the aggregation tracing during 10 minutes. Microscopic aggregates were also evaluated. After the PRP had been stirred for 10 minutes, a drop of the sample was placed on a slide and examined under a phase-contrast microscopy. A 0 to 4+ scale was used: 0, no aggregates; 1+, 1 to 3 aggregates composed of 2 to 5 platelets per aggregate per field (magnification 250); 2+, 4 to 5 aggregates composed of 6 to 10 platelets; 3+, 6 to 10 aggregates composed of 10 to 20 platelets; 4+, over 10 aggregates composed of more than 20 platelets per aggregate. The test was completed within three hours after blood collection and within one hour after preparing PRP. This test was performed on 68 patients (51 males, 17 females) with cerebral infarct (mean age of 61 years; range 32–86 years), 45 patients (34 males, 11 females) with TIA/RIND (mean age of 59 years; range 34–77 years), 11 patients (7 males, 4 females) with RVHD accompanied by cerebral embolism (mean age of 45 years; range 27–69 years) as well as the same number of age- and sex-matched normal and patient controls for each patient group.

Percentage of Megathrombocytes

Platelet size was measured with an ocular micrometer at a magnification of 1000 on stained peripheral
smears prepared from blood collected in EDTA. The percentage of platelets with diameter greater than 2.5 μ (megathrombocytes) was determined as follows: Platelets with a diameter of more than 2.5 μ were registered on a cell counter; 50 megathrombocytes were enumerated. This number served as the numerator. The total platelet counted served as the denominator for the determination of the percentage of megathrombocytes. This determination was obtained on 101 patients (74 males, 27 females) with cerebral infarct (mean age of 66 years; range 25–77 years), 76 patients (49 males, 27 females) with TIA/RIND (mean age of 61 years; range 36–75 years), 14 patients (9 males, 5 females) with RVHD accompanied by cerebral embolism (mean age of 48 years; range 24–69 years), 10 patients (5 males, 5 females) with RVHD not accompanied by cerebral embolism (mean age of 44 years, range 27–63 years) and the same number of age- and sex-matched controls for each group of patients. All subjects had normal platelet counts.

Since no significant difference in any test result between normal males and females was demonstrated, these were combined for analytical purposes.

Clinical Considerations

Results in each test were analyzed with respect to clinical course, severity, area of infarct by CT scan, and site of the lesion. In regard to clinical course, both horizontal and longitudinal studies were done. In the horizontal study, test results were compared with the time after onset of the stroke in each individual. In the longitudinal study, serial tests were carried out at 1, 2, and 4 days, and 1, 2, 3, and 4 weeks after onset of the clinical event in some individuals, to whom no anti-platelet agents were administered during this period. Patients with acute stroke were all admitted to the Neurological Institute of Tokyo Women’s Medical College and had varying degrees of neurological deficit and impairment of consciousness. The initial collection of blood was carried out within 24 hours after stroke in these patients. Clinical severity was established by the level of consciousness, the extension of lesion on CT scan and prognosis in cerebral infarct. In TIA and RIND, it was estimated by the extent of neurological deficit, the duration of symptoms and the frequency of episodes. The test results were also compared between carotid and vertebralbasilar system lesions or symptoms.

Effect of Aspirin

The effect of aspirin on the results of platelet function tests was studied. Serial tests were performed at 1, 2, and 4 weeks after starting oral aspirin (300 mg b.i.d.) in each subject. AIPA, RIPA, and SPA were determined in 48, 10, and 28 patients with TIA, RIND or cerebral infarct over four weeks after onset respectively. Percent megathrombocytes was determined in seven patients with TIA or cerebral infarct.

Results

Platelet Function Tests

AIPA was increased in the group of patients with cerebral infarct and TIA/RIND compared with the controls (table 1). In these patient groups, percent maximum aggregation was higher and irreversible aggregation was more frequently observed than in each control group. Although platelet aggregability is quite variable among normal subjects, the tendency toward increase in these patient groups was demonstrable in the present large population studies. In contrast, AIPA was not enhanced in the patients with RVHD accompanied by cerebral embolism. RIPA exhibited the same trend as AIPA (table 2).

Plasma factors, VIII:vWF and PAEF activities were both increased in the patients with obstructive CVD other than cerebral embolism resultant from RVHD (tables 3 and 4).

SPA was a frequent finding in the patients with TIA, RIND, and cerebral infarct (table 5). The normal range of percent maximum light transmittance in 100 normal subjects was 0 to 6.9% (mean ± 3SD) and microscopically positive SPA was 0 to 1+. Based on these data, we defined positive SPA by aggregometry as an increase in light transmission of over 7% with grossly visible platelet aggregates. Positive SPA by microscopy, on the other hand, was defined as the presence of 2+ aggregates in

<table>
<thead>
<tr>
<th>TABLE 1 Platelet Aggregation Induced By 2 μM ADP</th>
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<tr>
<td>Disease</td>
</tr>
<tr>
<td>Disease</td>
</tr>
<tr>
<td>Cerebral infarct</td>
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<tr>
<td>Normal control</td>
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<tr>
<td>Patient control</td>
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<tr>
<td>TIA/RIND</td>
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<tr>
<td>Normal control</td>
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<tr>
<td>Patient control</td>
</tr>
<tr>
<td>RVHD associated with cerebral embolism</td>
</tr>
<tr>
<td>Normal control</td>
</tr>
<tr>
<td>Patient control</td>
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</tbody>
</table>
Cerebral infarct: 24, 69.0 ± 16.8, < 0.001
Control: 24, 49.2 ± 14.9
TIA/RIND: 15, 64.9 ± 25.0, < 0.005
Control: 15, 48.0 ± 15.1
RVHD associated with cerebral embolism: 5, 45.7 ± 16.0, NS
Control: 5, 47.2 ± 14.6

A higher percentage of megathrombocytes was noted in patients with cerebral infarct and TIA/RIND than in the controls (table 6). The number of megathrombocytes in the patients with RVHD and cerebral embolism was elevated in the embolic but not the nonembolic subgroup. While the number of megathrombocytes was never elevated over 20% in normal controls, it was over 20% in 28 out of 45 (62%) patients with cerebral infarct and 36 out of 68 (46%) patients with cerebral embolism were found to have aggregometric or microscopic SPA. On the other hand, the SPA studies were negative in the patients with cerebral embolism and RVHD.

There was no significant difference in any test results between male and female patients.

Correlation with Clinical Course

Platelet aggregability was found to change significantly during the time course in 24 patients (14 males, 10 females) with acute cerebral infarct (mean age of 67 years; range 40–90 years) (fig. 1). Within the first four days, it was within normal limits (x ± SD = 42.5 ± 10.8 in the same number of age- and sex-matched controls); after a week it began to increase significantly (p < 0.05) and continued to increase even after four weeks from onset. While figure 1 shows the mean time course of AIPA in the 24 patients, individual time courses were also similar to this in the majority of the patients. The increase of AIPA in any day within the first four days was observed only in 4 out of 24 (16.7%) patients. Also in the horizontal studies, the same tendency was observed as in the longitudinal studies.

While megathrombocytes were increased over 20% in one-fourth of the patients with cerebral infarct (table 6), they were not increased in 12 acute cases within three days after onset (x² = 4.26; p < 0.05). Serial longitudinal studies independent of these observations were done in five patients with acute cerebral infarct (mean age of 66 years; range 37–78 years) (fig. 2). The percentage of megathrombocytes was below 20% in all cases within 48 hours after onset and it began to increase gradually in a week; thereafter it slightly decreased again or continued to increase over several weeks. In the patients with TIA, we did not observe a significant correlation of the percent megathrombocytes or AIPA with the time after the episode. There was no evident correlation with clinical course in any other platelet function test.

Correlation with Clinical Severity

The presence of SPA was correlated with the frequency of TIA. SPA was far more frequently observed in patients with multiple TIA (15 out of 16, 94%) than in patients with single TIA (8 out of 19, 42%) (x² = 10.35; p < 0.005). SPA was not highly correlated with the extent or duration of neurological manifestations. There was no significant correlation of the results of any other test with clinical severity. There was no difference in the result of any test in any patient group whether carotid or vertebrobasilar system was affected.

Effect of Aspirin on Test Results (Table 7)

AIPA, RIPA, and SPA returned to normal after one week of aspirin treatment. Percent megathrombocytes was reduced after two and four weeks of aspirin treatment. There was no significant difference in response of any test to aspirin between male and female patients.

Discussion

Platelets are a major component of early arterial thrombi. We have previously observed increased platelet aggregation in patients with thrombotic CVD as reported by others.1–7 In the present study we also
VIII:vWF is essential during the course of acute stroke. Platelet aggregation was not increased within the first four days in the majority of our patients with acute cerebral infarct. This early absence of platelet hyperaggregability has not been observed in several other studies although O'Brien noted it and postulated that it could be due to an increased number of "empty exhausted platelets" which undergo release during thrombogenesis but continue to circulate for a normal time.

In confirmation of a previous report, the plasma level of VIII:vWF was increased as well as the enhancement of RIPA, which requires VIII:vWF, in the patients with thrombotic CVD. VIII:vWF is essential for platelet adhesion to the subendothelium. PAEF has been also increased in these patients. PAEF has been reported increased in patients with diabetic vasculopathy by some and not by other authors. Whether increase of these factors plays a role in the initiation of thrombotic events in these patients is not known.

SPA has been reported in various thrombotic disorders. We observed SPA in a high percentage of the patients with thrombotic CVD. Among the tests performed, only the incidence of SPA was correlated with the frequency of TIA. SPA appears to reflect the fragility of platelets to mechanical forces.

The large platelet or megathrombocyte is a young platelet recently released from the bone marrow. It has been used to predict thrombocytolytic states either decompensated or compensated, where the platelet count is normal but the platelet survival is shortened, indicating increased platelet turnover. Megathrombocytes were mildly but significantly increased in our patients with thrombotic CVD. This finding suggests increased platelet turnover in CVD, developing in the patients with acute cerebral infarct after three days from onset and increasing after a week. The reduction of percent megathrombocytes following the oral intake of aspirin is considered to result from the inhibition of accelerated platelet consumption, which is a consequence of the prevention of intravascular platelet aggregation by this drug.

In the patients with cerebral embolism of cardiac origin and RVHD, AIPA, and RIPA were not increased, SPA was not observed, and VIII:vWF and VIII:vWF are essential during the course of acute stroke. Platelet aggregation was not increased within the first four days in the majority of our patients with acute cerebral infarct. This early absence of platelet hyperaggregability has not been observed in several other studies although O'Brien noted it and postulated that it could be due to an increased number of "empty exhausted platelets" which undergo release during thrombogenesis but continue to circulate for a normal time.

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PAEF were not increased in comparison with the patients with other types of ischemic CVD. This difference suggests that the platelet hyperaggregability and the increase of plasma factors in patients with thrombotic CVD are at least not the result of arterial obstruction. The percentage of megathrombocytes was increased in the embolic but not the non-embolic patient with RVHD. This may indicate that a larger amount of thrombocytolysis and/or thrombus formation occurs around the abnormal valves in the embolic group than in the non-embolic group.

There are a large number of platelet function tests that characterize different steps in the spectrum of platelet reactions. As no single test has yet proven adequate in determining the overall functional status of platelets, we selected for study several tests which have been properly standardized, do not require special equipment and can be repeated frequently. Platelet count, skin bleeding time and platelet retention also satisfy these conditions, although these studies were negative in this series of patients.

On the basis of the present study, hyperaggregable platelets, accompanied by an increase of plasma activators of platelet function, exist in thrombotic CVD, but not in patients with RVHD and cerebral embolism. These findings suggest that the systemic abnormalities described in the thrombotic CVD are related to its pathogenesis. Since these abnormalities were not observed in the embolic patients with RVHD, it is presumed that local abnormalities including hemodynamic alterations may be more important factors in the pathogenesis of embolism.

American and Canadian prospective cooperative clinical studies of antiplatelet therapy in patients with TIA suggest that platelets play a primary role in the pathogenesis of TIA. In the American study, aspirin was found to be effective in reducing the frequency of TIA and also in improving prognosis in patients with TIA due to stenotic lesions in the carotid system. In the Canadian study, mortality rate at 42 months after medication was reduced by one half in aspirin-treated male patients. No explanation could be found from our study why aspirin should be effective only in males in the Canadian study, since we observed no sex difference in platelet abnormalities or in their reversal by aspirin. On the basis of the present study, we suggest that: 1) platelet abnormalities play a role in the pathogenesis of thrombotic strokes; 2) platelet-suppressive agents should be administered in patients with thrombotic CVD and normal platelet function, and 3) the clinical and laboratory effects of such treatment should be compared with those in patients with thrombotic CVD and abnormal platelet function. Results of such a study should help clarify further the role of platelets in the etiology of stroke in individual patients.

Acknowledgement

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References


Table 7 Effect of Aspirin on Platelet Function Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Number</th>
<th>Before treatment</th>
<th>1 week</th>
<th>2 weeks</th>
<th>4 weeks</th>
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<tbody>
<tr>
<td>ADP-induced platelet aggregation</td>
<td>48</td>
<td>59.6±14.0%</td>
<td>39.2±6.1%†</td>
<td>38.5±4.9%†</td>
<td>40.1±7.2%†</td>
</tr>
<tr>
<td>Ristocetin-induced platelet aggregation</td>
<td>10</td>
<td>72.5±10.3%</td>
<td>32.9±13.6%†</td>
<td>34.2±9.9%†</td>
<td>34.6±10.3%†</td>
</tr>
<tr>
<td>Spontaneous platelet aggregation</td>
<td>28</td>
<td>15 (53%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% Megathrombocytes</td>
<td>17</td>
<td>19.0±5.6%</td>
<td>18.1±5.0%</td>
<td>15.1±4.1%*</td>
<td>14.9±4.7%*</td>
</tr>
</tbody>
</table>

*p < 0.05.
†p < 0.001.

FIGURE 2. Changes in the percentage of megathrombocytes in five patients with acute cerebral infarct.
Platelet function tests in thrombotic cerebrovascular disorders.
S Uchiyama, M Takeuchi, M Osawa, I Kobayashi, S Maruyama, M Aosaki and K Hirosawa

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