Platelet Volume, Aggregation, and Adenosine Triphosphate Release in Cerebral Thrombosis

Hideo Tohgi, MD; Hajime Suzuki, MD; Kenichi Tamura, MD; and Bunsho Kimura, MD

We compared whole blood platelet aggregation, adenosine triphosphate release, platelet count, platelet crit (percentage volume of platelets), and mean platelet volume during the acute, subacute, and chronic periods of cerebral thrombosis in 22 patients with values in 29 controls. During the acute and subacute periods, platelet aggregation, platelet count, platelet crit, and mean platelet volume were significantly less in the patients than in the controls \((p<0.05-0.01)\) while the adenosine triphosphate release rate per volume of platelets was significantly greater \((p<0.05)\). During the acute period, infarct size showed a significant positive correlation with platelet aggregation \((r=0.59, p<0.01)\) and adenosine triphosphate release rate \((r=0.70, p<0.001)\) but a negative correlation with platelet count \((r=-0.44, p<0.05)\). Our results suggest that platelet aggregation is reduced during the acute period due to the consumption of platelets during thrombogenesis but that the remaining individual platelets are hyperactive. Platelet consumption during the acute period increases with infarct size. During the chronic period, platelet crit and mean platelet volume were significantly less in the patients than in the controls \((p<0.01)\) while the adenosine triphosphate release rate was significantly greater \((p<0.01)\), suggesting sustained platelet consumption and chronically enhanced secretion of individual platelets. 

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Platelet aggregation plays a central role in the pathogenesis of thromboembolic cerebrovascular disease. It is still controversial, however, whether platelets are hyperactive, unchanged, or hypoactive during the acute and chronic periods of ischemic stroke and whether the change in platelet activity is the primary event in the pathogenesis of ischemic stroke. While some previous studies using platelet aggregation, circulating platelet aggregates, or platelet marker proteins (i.e., platelet factor 4, β-thromboglobulin) as indicators of platelet function have shown platelet hyperactivity during the acute period of stroke, a few other studies have reported that platelet aggregation is not enhanced during the acute period but is increased several days after the stroke. The lack of platelet hyperaggregability during the acute period has been ascribed to the consumption of active platelets during thrombogenesis.

Increased platelet activity has also been shown during the chronic period of stroke by measuring platelet aggregation and platelet adhesiveness. In contrast, a trend toward a lower percentage spontaneous platelet aggregation has been reported during the subacute and chronic periods, particularly in patients with angiographic evidence of atherosclerosis. Such inconsistent results may be due to differences in the methods of evaluating platelet function, the heterogeneity of patients in regard to stroke subgroups, and the interval after stroke onset.

In thrombotic patients with lacunar infarcts in areas of the perforating arteries, we serially measured whole blood platelet aggregation and the release of adenosine 5'-triphosphate (ATP) from the acute period to the chronic period using a whole blood lumiagregometer, which enabled us to make the measurements in the presence of all blood elements, as in the in vivo state, without blood centrifugation. Furthermore, we correlated platelet function variables during the acute period with the size of the infarct.

Subjects and Methods

We studied 22 patients with cerebral thrombosis (age 43–83, mean±SD 64.0±12.9 years) and 29 controls (age 43–81, mean±SD 55.3±9.5 years). The patients were studied serially, during the acute (≤7 days), subacute (2–3 weeks), and chronic (4–5 weeks) periods after the stroke. The controls were healthy individuals who had not received any drug. Six controls had hypertension, and 10 smoked. Eight patients had hypertension, two had diabetes mellitus,
and five smoked. In both patients and controls, we excluded subjects with a history of stroke, cardiac disease, occlusive peripheral vascular disease, hypercholesterolemia, excessive smoking, and alcohol consumption. We also excluded patients with malignant or infectious diseases that may alter platelet function. Informed consent was obtained from all subjects.

Since there were no significant age-related changes or smoker–nonsmoker differences in any platelet function variable measured, we did not divide the subjects with regard to age or smoking. There were no significant differences in hematocrit between the controls (40.2±3.3%) and the patients during the acute (41.5±3.7%), subacute (39.4±5.4%), or chronic (41.5±3.8%) periods, which enabled us to rule out effects of hemoconcentration or hemodilution on the results. During the acute period the patients were treated with 400–600 ml/day of 10% glycerol and/or 500 mg/day of dextran 40. We measured platelet function variables for the acute period immediately after admission, before the infusion of glycerol and/or dextran, to exclude any possible interference of the agents with platelet function.16,17 Heparin, warfarin, and fibrinolytic agents were not administered throughout the study. No patient had received antiplatelet medication before or after the stroke.

Blood was withdrawn from an antecubital vein into a plastic syringe containing one-tenth volume of 3.8% (0.13 M) trisodium citrate. Platelet aggregation and ATP release were measured simultaneously using a whole blood lumiaaggregometer12–14 (model 560, Chrono-Log Corp., Havertown, Pa.). Aggregation was induced by collagen (Chrono-Log Corp.) with a final concentration of 2 μM; 40 μl of luciferin-luciferase reagent (Chronolume, Chrono-Log Corp.) was added to each 960-μl sample 30–60 seconds before the addition of collagen.

Platelet aggregation was measured 6 minutes after the addition of collagen as the increase in impedance across a pair of electrodes placed in the blood sample relative to a standard (5 Ω) impedance. Luminescence due to ATP released during platelet aggregation was measured relative to the luminescence induced by 3 μmol standard ATP (Sigma Chemical Co., St. Louis, Mo.). Platelet count, mean platelet volume, and platelet crit (percentage volume of platelets, mean platelet volume x platelet count) were measured using a Sysmex E-4000 device (Toa Medical Electronics Co. Ltd., Kakogawa, Japan).

Computed tomography was performed in the patients on admission and 1 or 2 weeks thereafter. Size of the infarct was calculated as maximum length x maximum width of the hypodense area on the horizontal computed tomograms in which the area appeared largest.

We expressed the results as mean±SD and compared the groups by using Student's t test. We calculated the correlation coefficients between infarct size and platelet function variables as the Pearson product-moment correlation.

Results

In the controls, platelet aggregation was not correlated with platelet count, mean platelet volume, or platelet crit, whereas ATP release was significantly correlated with platelet count (range 15–40×10³/ mm³) (r=0.40, p<0.05) and platelet crit (range 0.16–0.39%) (r=0.41, p<0.05; Figure 1) but not with mean

![Figure 1. Scatter plot of relation between platelet crit (mean platelet volume x platelet count) and release of adenosine 5'-triphosphate (ATP) in control subjects.](image)
TABLE 2. Correlation Coefficients Between Infarct Size and Platelet Function Variables During Acute and Chronic Periods in Stroke Patients

<table>
<thead>
<tr>
<th>Platelet function variable</th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregation</td>
<td>0.59*</td>
<td>0.33</td>
</tr>
<tr>
<td>ATP release</td>
<td>0.39</td>
<td>0.34</td>
</tr>
<tr>
<td>ATP release rate</td>
<td>0.70†</td>
<td>0.08</td>
</tr>
<tr>
<td>Platelet count</td>
<td>-0.44‡</td>
<td>0.39</td>
</tr>
<tr>
<td>Platelet crit</td>
<td>-0.42</td>
<td>0.44</td>
</tr>
<tr>
<td>Mean platelet volume</td>
<td>-0.06</td>
<td>0.07</td>
</tr>
</tbody>
</table>

ATP, adenosine 5'-triphosphate.
*†‡p<0.01, 0.001, and 0.05, respectively, different from 0.

platelet volume (range 9.5-12.2 fl) \( r = -0.03 \). Therefore, we calculated the ATP release rate per volume of platelets as moles per liter. Platelet aggregation had a significant positive correlation with ATP release \( r = 0.39, p < 0.05 \) and ATP release rate \( r = 0.46, p < 0.02 \).

Table 1 shows platelet aggregation, ATP release, ATP release rate, platelet count, platelet crit, and mean platelet volume in the controls and in the patients during the acute, subacute, and chronic periods. During the acute period platelet aggregation was significantly less in the patients than in the controls \( p < 0.05 \); platelet aggregation then increased progressively in the patients until it was no longer different from the control value during the chronic period. In the patients ATP release showed a tendency to be less than control during the acute period but was significantly greater during the chronic period than during the acute period \( p < 0.05 \). The ATP release rate was significantly greater in the patients during all periods than in the controls \( p < 0.01-0.05 \). Platelet crit and mean platelet volume were significantly less in the patients during all periods than in the controls.

Table 2 shows the correlation coefficients between infarct size (range 0–1.2 cm³) and the platelet function variables, platelet count, and platelet crit during the acute and chronic periods. During the acute period, infarct size showed a significant positive correlation with platelet aggregation \( r = 0.59, p < 0.01 \) (Figure 2, top) and ATP release rate \( r = 0.70, p < 0.001 \) (Figure 2, center) while there was a significant negative correlation between infarct size and platelet count \( r = -0.44, p < 0.05 \) (Figure 2, bottom). Infarct size was not correlated with mean platelet volume. There was no significant relation between infarct size and any platelet function variables during the chronic period.

Discussion

Our results on the acute phase of cerebral thrombosis show significant reductions in collagen-induced whole blood platelet aggregation, platelet count, platelet crit, and mean platelet volume and a significant increase in the ATP release rate. The decreased platelet aggregation shown in this study is at variance with the findings of some previous studies that have reported hyperaggregability of platelets during the acute period of stroke. In other studies, however, platelet aggregation was not increased or decreased within the first few days after stroke. The decrease in whole blood platelet aggregation is not explained by the decrease in platelet count of approximately \( 5 \times 10^4 \text{mm}^3 \) on average. Nor is it ascribable to an increase in the number of "empty exhausted platelets" which have undergone release reaction during thrombogenesis because the ATP release rate was significantly increased. Furthermore, we
found that infarct size correlates positively with platelet aggregability during the acute period. Although we do not know platelet aggregability prior to the stroke, one speculation is that patients with increased platelet aggregability before a stroke develop large infarcts in areas of the perforating arteries.

In contrast to the decrease in platelet aggregability, we found that the ATP release rate is enhanced during any period of cerebral thrombosis compared with the controls, which is in keeping with the results of Joseph et al. These findings indicate that collagen induces only direct or aggregation-independent platelet activation and that dense body secretion may occur independently of aggregation. While previous results on platelet aggregation based upon the light transmission method have varied, studies on platelet release of β-thromboglobulin, platelet factor 4, and serotonin have consistently demonstrated platelet hyperactivation in acute stroke patients. Shah et al found elevated levels of β-thromboglobulin and platelet factor 4 in patients with thrombomembolic and cardioembolic stroke, unrelated to infarct size, but not in those with minor strokes (lacunar stroke and transient ischemic attacks). In our study, ATP release rate was elevated during the acute period of lacunar stroke and increased with infarct size. These differences in results may reflect the differences in stroke pathophysiology between ATP released from dense granules and that released from α-granule proteins.

Our results show significant decreases in platelet count and platelet crit during the acute period of stroke, similar to some previous findings. The decrease in platelet count has been generally unnoticed in previous reports, presumably because it may have been masked by hemoconcentration, which is often seen during the acute period of severe stroke. We excluded the possibility of hemodilution because there were no significant differences in hematocrit between the patients and controls. Although we have no direct evidence to explain the reduced platelet count, the significant decrease in mean platelet volume during all periods of stroke may reflect a selective loss of large, active platelets since large platelets containing more adenosine nucleotide aggregate earlier than small platelets. Previous studies have demonstrated an increased ratio of circulating platelet aggregates during the acute period of cerebral infarction and transient ischemic attacks. These findings suggest that platelet consumption occurs during the acute period of cerebral ischemia. If this is the case, large platelets presumed to have aggregated may have an even greater capacity for ATP release than our measurements indicate. However, the decrease in platelet count by approximately 5 × 10^11/mm^3 on average is not ascribable to thrombogenesis in cerebral arteries responsible for the lacunar infarcts alone; the decrease may reflect hyperaggregability occurring systemically in stroke patients. A significant negative correlation between platelet crit during the acute period and infarct size suggests that more platelets have been consumed in patients with larger infarcts, although there was no correlation between infarct size and mean platelet volume. However, it is generally thought that enhanced platelet consumption in most acute thrombotic processes is usually not associated with a decrease in the platelet count. Further studies on platelet kinetics are needed to evaluate the significance of our results.

Platelet function during the chronic period of cerebral infarction has been shown to be increased by some authors but not others. In our study, platelet aggregation and ATP release in patients during the chronic period were not different from those in the controls. However, the ATP release rate was significantly increased and platelet crit and mean platelet volume were significantly decreased during the chronic period, suggesting chronically enhanced secretion of individual platelets and chronic platelet consumption. This hypothesis is supported by the known lower percentage spontaneous platelet aggregation, decreased platelet survival time, and increased percentage megathrombocytes or large platelets, which indicates a thrombocytolytic state and enhanced platelet turnover. These findings should have implications in predicting the risk of stroke recurrence and in taking preventive measures.

Acknowledgments

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