Shear-Induced Platelet Aggregation in Cerebral Ischemia

Shinichiro Uchiyama, MD; Masako Yamazaki, MD; Shoichi Maruyama, MD; Makoto Handa, MD; Yasuo Ikeda, MD; Mayumi Fukuyama; Ichiro Itagaki

Background and Purpose
Recent evidence has suggested that shear-induced platelet aggregation is an important mechanism of thrombosis at arterial bifurcations or stenoses. We measured shear-induced platelet aggregation with a new apparatus and studied platelet aggregation (SIPA) in patients with cerebral ischemia and also studied correlations with other hemostatic parameters as well as the effect of antiplatelet agents.

Methods
The subjects were 75 patients with cerebral ischemia and 26 control subjects. Platelet aggregation was induced in citrated platelet-rich plasma by a high shear stress (108 dynes/cm²) that was applied by means of a cone-plate streaming chamber based on turbidimetry. We studied the correlation of test results with hemostatic parameters and also the effects of antiplatelet agents.

Results
Compared with the control subjects, an increase of shear-induced platelet aggregation was observed in 21 patients with atherothrombotic stroke and 12 with transient ischemic attacks, but not in 11 with cardioembolic stroke or 31 with lacunar stroke. There was no significant correlation of shear-induced platelet aggregation with platelet count, agonist-induced platelet aggregation, fibrinogen level, or β-thromboglobulin level. The extent of shear-induced aggregation was not correlated with von Willebrand factor antigen levels but was significantly correlated with the amounts of larger von Willebrand factor multimers. Oral aspirin (81 mg/d) did not inhibit shear-induced platelet aggregation, whereas oral ticlopidine (200 mg/d) significantly inhibited it.

Conclusions
These results indicate that shear-induced platelet aggregation is increased in patients with atherothrombotic stroke and transient ischemic attacks, is correlated with the increase of larger von Willebrand factor multimers, and is corrected by ticlopidine but not by low-dose aspirin. (Stroke. 1994;25:1547-1551.)

Key Words  • cerebral ischemia • platelet aggregation • thrombosis • ticlopidine

Platelet activation has an important role in arterial thrombosis and can be induced not only by physiological agonists but also by physical shear stress. Deposition of platelet aggregates in the arterial circulation occurs preferentially at sites of bifurcation, sharp bend, and stenosis, where flow separation is likely to occur. In vitro experiments have shown that fluid shear stress may promote platelet aggregation by direct activation or by enhancing the response to chemical stimuli. This mechanism may be of great importance for thrombogenesis in patients with ischemic stroke or transient ischemic attacks (TIA), who develop thrombi in the arteries of the neck or the brain. Spontaneous platelet aggregation or rheological platelet aggregation induced in vitro by shearing forces alone has occasionally been found in patients with various atherothrombotic diseases.3-5

In the present study we measured shear-induced platelet aggregation (SIPA) in patients with cerebral ischemia with a newly developed apparatus and studied the correlation of SIPA with other hemostatic parameters as well as the influence of antiplatelet agents.

Subjects and Methods
The subjects were 63 patients with cerebral infarction (41 men and 22 women; mean age, 65 years range, 30 to 87 years) and 12 patients with TIA (7 men and 5 women; mean age, 65 years [range, 49 to 83 years]), who were studied within 3 weeks of the onset of illness and had not been given any antiplatelet agents, anticoagulants, or fibrinolytic agents. In addition, we studied 14 healthy nonsmoking volunteers (6 men and 8 women; mean age, 31 years [range, 27 to 42 years]) and 12 nonsmoking patients with various neurological disorders except for stroke and without hypertension, diabetes mellitus, or hyperlipidemia (7 men and 5 women; mean age, 60 years [range, 48 to 75 years]).

Cerebral infarction was classified as atherothrombotic, cardioembolic, or lacunar based on the clinical manifestations and the findings of brain computed tomography, brain magnetic resonance imaging, cerebral angiography, electrocardiography, and echocardiography, according to the Classification of Cerebrovascular Diseases III of the National Institute of Neurological Disorders and Stroke. Atherothrombotic infarction was diagnosed when there was evidence of arterial stenotic plaque or occlusion at one or more sites in the extracranial and major intracranial arteries along with the absence of potential cardiac sources of embolism. A diagnosis of cardioembolic infarction was made on the basis of evidence of a potential cardiac source of embolism, a rapid onset of symptoms, evidence of multiple brain or systemic infarcts, and the absence of atherosclerotic lesions in the large arteries. Lacunar infarction was defined as small, deep subcortical infarcts (<1.5 cm in diameter) in the territory of the perforating arteries in patients without large arterial lesions or potential cardiac sources of embolism.26 Patients who did not meet

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the criteria for any of the above three clinical categories were excluded from this study. Patients with primary coagulation disorders including antiphospholipid antibody syndrome, hereditary deficiencies of coagulation inhibitors, abnormalities of fibrinolysis, thrombocytosis, and polycythemia were also excluded.9

Fig 1 shows a diagram of the apparatus used to measure SIPA.16 Our method for the continuous monitoring of SIPA was based on a turbidimetric technique that uses a thermostatted cone-plate streaming chamber made of polymethylacrylate. The light beam from a helium-neon laser was passed through a sample of platelet-rich plasma in the chamber to the photodetector, and sequential changes in the light transmittance resulting from platelet aggregation due to shear stress caused by rotation of the cone were displayed with a computer system.

Platelet aggregation was also determined in response to 2 μmol/L ADP (Niko Bioscience), 0.42 mmol/L arachidonic acid (AA) (Nakarai Kagaku), and 0.2 μmol/L platelet-activating factor (PAF) (Funakoshi Yakuhin) according to the method described previously.12 In addition, the β-thromboglobulin (β-TG) level was determined in plasma separated by centrifugation at 1500g for 30 minutes at 4°C from 2.5 mL of venous blood collected into an ice-cold Diatube-H (Diagnostica Stago) containing 0.3 mL of anticoagulants including sodium citrate, citrate, theophylline, adenosine, and dipyridamole. The plasma sample was subjected to enzyme-linked immunosorbent assay using an Asserachrom β-TG kit (Diagnostica Stago).

Platelet aggregation was determined in 26 control subjects and 75 patients with cerebral ischemia. SIPA was determined at both 12 and 108 dynes/cm² in all subjects. However, the platelet aggregation induced by low shear stress (12 dynes/cm²) proved to be unstable and quite variable, since it was greatly affected by the time elapsed after blood collection. Therefore, only platelet aggregation induced by high shear stress (108 dynes/cm²) was analyzed in this study. SIPA at 108 dynes/cm² was determined on different days in 8 normal volunteers, and there was no significant difference between the first and second determinations (the mean±1 SD of the first and second determinations was 48.6±9.7% and 49.9±6.8%, respectively; P=.54). There was no significant correlation between SIPA and the age of either the control
subjects or the patients with cerebral ischemia, and there were no significant differences between men and women. There was also no significant correlation between SIPA and the duration after onset in the patients with cerebral ischemia. SIPA was increased in the 21 patients with atherothrombotic stroke and the 12 TIA patients compared with normal or patient control subjects, but it was not increased in the 11 patients with cardioembolic stroke or the 31 patients with lacunar stroke (Table 1). In addition, SIPA was significantly higher in the patients with atherothrombotic stroke or TIA than in the patients with cardioembolic stroke or lacunar stroke. There was no significant correlation between SIPA and platelet count or platelet aggregation in response to ADP, AA, or PAF, as well as no correlation between fibrinogen or β-TG levels and SIPA (Table 2).

There was no significant correlation between SIPA and plasma vWF antigen levels in the 26 patients for whom both were determined simultaneously (Table 2), whereas SIPA was likely to be increased in the patients with dense bands of larger vWF multimers (Fig 2). Larger multimers were defined as bands above the 10th band from the bottom, indicating molecular weights of >13 to 14X10^6. SIPA indicates shear-induced platelet aggregation.

248±77 arbitrary units) than in 12 patients with lacunar stroke (147±67 U) (P<.001) or in 5 patient control subjects (114±43 U) (P<.01). There was no significant difference in the level of larger vWF multimers between 4 TIA patients (156±91 U) and patients with atherothrombotic or lacunar stroke or patient control subjects.

The effects of two antiplatelet agents on SIPA were also studied in the patients with cerebral ischemia. Oral aspirin (81 mg/d) did not affect SIPA, whereas oral ticlopidine (200 mg/d) clearly inhibited it (Fig 4).

**Discussion**

Recent evidence has suggested that SIPA is an important mechanism of thrombogenesis at sites of arterial bifurcation or stenosis. Fluid shear stress in arteries and arterioles stenosed by atherosclerosis or spasm may exceed the normal average level of 20 dynes/cm². In vitro platelet aggregation begins to occur when a fluid shear stress of at least 30 to 60 dynes/cm² is applied for 30 seconds. Regarding the molecular mechanism of SIPA, it is known that the interaction of fibrinogen with glycoprotein (GP) IIb/IIIa is required at low shear stresses, while interaction of vWF with both GP Ib and GP IIb/IIIa is required at high shear stresses.

Platelet aggregation induced by a high shear stress of 108 dynes/cm² was increased in our patients with atherothrombotic stroke and TIA but not in those with cardioembolic or lacunar stroke. These differences in SIPA between subtypes of stroke may reflect differences in the pathogenesis of the underlying condition. Atherothrombotic stroke was calculated by means of densitometry with dense bands of larger vWF multimers (Fig 2). Whereas SIPA was likely to be increased in the patients whom both were determined simultaneously (Table 2), there were no significant differences between men and women. There was also no significant correlation between SIPA and the duration after onset in the patients with cerebral ischemia. SIPA was increased in the 21 patients with atherothrombotic stroke and the 12 TIA patients compared with normal or patient control subjects, but it was not increased in the 11 patients with cardioembolic stroke or the 31 patients with lacunar stroke (Table 1). In addition, SIPA was significantly higher in the patients with atherothrombotic stroke or TIA than in the patients with cardioembolic stroke or lacunar stroke. There was no significant correlation between SIPA and platelet count or platelet aggregation in response to ADP, AA, or PAF, as well as no correlation between fibrinogen or β-TG levels and SIPA (Table 2).

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**Table 1. Shear-Induced Platelet Aggregation in Subtypes of Cerebral Ischemia**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>n</th>
<th>SIPA, % (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherothrombotic stroke</td>
<td>21</td>
<td>57.9±10.4</td>
</tr>
<tr>
<td>Cardioembolic stroke</td>
<td>11</td>
<td>48.3±11.8</td>
</tr>
<tr>
<td>Lacunar stroke</td>
<td>31</td>
<td>49.2±8.1</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>12</td>
<td>57.5±8.6</td>
</tr>
<tr>
<td>Patient control</td>
<td>12</td>
<td>46.3±10.3</td>
</tr>
<tr>
<td>Normal control</td>
<td>14</td>
<td>44.9±2.7</td>
</tr>
</tbody>
</table>

*P<.05, †P<.01, ‡P<.001 (ANOVA).

**Table 2. Correlation Between Shear-Induced Platelet Aggregation and Other Hemostatic Parameters in Patients With Cerebral Ischemia**

<table>
<thead>
<tr>
<th>Hemostatic Parameters</th>
<th>n</th>
<th>Range</th>
<th>Mean±SD</th>
<th>Correlation Coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count, x10^3/mm³</td>
<td>66</td>
<td>11.4-54.6</td>
<td>31.9±10.4</td>
<td>.228</td>
<td>.097</td>
</tr>
<tr>
<td>Platelet aggregation, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced by ADP</td>
<td>45</td>
<td>5-82</td>
<td>49±21</td>
<td>.203</td>
<td>.242</td>
</tr>
<tr>
<td>Induced by AA</td>
<td>45</td>
<td>2-86</td>
<td>69±14</td>
<td>.239</td>
<td>.168</td>
</tr>
<tr>
<td>Induced by PAF</td>
<td>45</td>
<td>3-85</td>
<td>42±26</td>
<td>.170</td>
<td>.330</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>29</td>
<td>127-726</td>
<td>335±159</td>
<td>.121</td>
<td>.533</td>
</tr>
<tr>
<td>β-Thromboglobulin, ng/mL</td>
<td>27</td>
<td>14-360</td>
<td>65±79</td>
<td>.112</td>
<td>.578</td>
</tr>
<tr>
<td>vWF antigen, U</td>
<td>26</td>
<td>0.06-2.18</td>
<td>1.32±0.49</td>
<td>.079</td>
<td>.707</td>
</tr>
</tbody>
</table>

AA indicates arachidonic acid; PAF, platelet-activating factor.
stroke and TIA mainly occur when platelet-rich thrombi form on atheromatous plaques or ulcers in large arteries, whereas lacunar strokes are deep and small infarcts that are caused by arteriosclerosis of small perforating arteries. In a study using $^{111}$In-labeled platelets we previously found a reduction of platelet survival time and accumulation of platelets in patients with atherothrombotic stroke but not in those with lacunar stroke. Therefore, atherothrombotic stroke may be a more platelet-dependent disease state than lacunar stroke, at least in a quantitative sense. In contrast, since cardioembolic stroke is attributable to fibrin-rich thrombi formed in the heart chamber, this subtype of stroke may not have much platelet dependence.

We previously found an increase of platelet aggregation in response to ADP, AA, and PAF in patients with cerebral ischemia, as well as an increase of β-TG and platelet factor 4. These findings suggested the presence of platelet activation associated with arterial thrombosis in such patients. The increase of SIPA noted in the present study provides additional evidence of platelet activation in patients with atherothrombotic stroke and TIA. However, there was no significant correlation of SIPA with platelet aggregation stimulated by ADP, AA, or PAF or with β-TG level. Therefore, the increase of SIPA appears to reflect a different aspect of platelet activity from agonist-induced platelet aggregation or from in vivo secretion of α-granules.

Although there was no correlation between the extent of SIPA and the level of vWF antigen, a significant correlation was observed between SIPA and the level of larger vWF multimers, which are known to play a major role in platelet aggregation at high shear stresses. Moake et al determined that full and irreversible aggregation can only be induced by a high shear stress (120 dynes/cm$^2$) applied for 30 seconds when large vWF multimeric forms are present, and that unusually large vWF multimers of the type produced by human endothelial cells are optimally effective. These large or unusually large vWF multimer forms must bind to both GP Ib and the GP IIb/IIIa complex on the platelet membrane for SIPA to occur. The results obtained in the present study suggest that an increase of the plasma level of larger vWF multimers is one possible mechanism for an increase of SIPA in patients with cerebral ischemia. Indeed, the larger vWF multimer level was significantly higher in atherothrombotic patients than in lacunar patients or patient control subjects. The reason for this remains unclear, although it is possible that an increase of larger vWF multimers was associated with the presence or the extent of atherosclerosis. For example, atherosclerosis might alter endothelial function and activate platelets, which might in turn increase the release of vWF from endothelial cells and platelets themselves.

We found that SIPA was unchanged by treatment with 81 mg of aspirin. We previously demonstrated that this dose was sufficient to suppress both platelet aggregation induced by AA and thromboxane (TX) A$_2$ formation. Failure of inhibition by low-dose aspirin may reflect the lesser importance of TXA$_2$ in the mechanism of SIPA, as was suggested in previous in vitro experiments which showed that neither a cyclooxygenase inhibitor (indomethacin) or a TXA$_2$ synthetase inhibitor (CV-4151) could inhibit SIPA. Moake et al also reported that the treatment of platelets with aspirin in vivo and in vitro had no inhibitory effect on SIPA. On the other hand, it has been reported that high-dose aspirin inhibits SIPA. Ratnatunga et al reported that ingestion of a single 600-mg aspirin tablet significantly inhibited SIPA and concluded that aspirin can affect the platelet response to shear forces at doses higher than 300 mg, suggesting a mechanism probably different from that of interference with TXA$_2$ formation.

SIPA was inhibited by treatment with 200 mg of ticlopidine. This is the usual dosage in Japan, and we have previously shown that this dose was sufficient to suppress platelet aggregation induced by ADP in the average Japanese patient. Ticlopidine is known to be a specific inhibitor of ADP-dependent platelet aggregation, and recent studies have suggested that ticlopidine acts by preventing the inhibition of adenylate
cyclohexyl-ADP through an effect on the ADP receptor-Gi protein complex. Previous studies have demonstrated that ADP released from the dense granules of platelets is capable of inducing the binding of vWF to the GPIb/IIIa complex on the platelet membrane and that SIPA mediates by larger vWF multimers released from platelets or endothelial cells requires the presence of ADP. Based on these studies, it appears that ticlopidine can inhibit vWF-dependent platelet aggregation induced by high shear stresses, which requires ADP as a cofactor, by inhibiting the ADP-dependent receptor mechanism for the binding of vWF to the platelet membrane.

In conclusion, the present study indicates that SIPA is increased in patients with atherothrombotic stroke and TIA, that this increase is correlated with an increase of large vWF multimers, and that it is corrected by ticlopidine but not by low-dose aspirin. SIPA appears to be a useful parameter for evaluating the abnormal rheological properties of platelets and the effects of pharmacological agents. It may be important to investigate correlations between the inhibition of SIPA and the improvement of clinical symptoms in various atherothrombotic diseases in the future.

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References

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