Platelet Aggregability Measured by Screen Filtration Pressure Method in Cerebrovascular Diseases

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SUMMARY Platelet aggregability was measured using the screen filtration pressure (SFP) method in 50 elderly healthy people, 93 persons with essential hypertension, 166 patients with cerebral thrombosis, and 74 patients with cerebral hemorrhage at the recovery stage. SFP by 3 μM ADP in the healthy persons, the hypertensive patients, and the patients in the recovery stages of hemorrhage and thrombosis were 148.7 ± 53.5, 176.2 ± 74.4, 189.8 ± 58.3 and 206.3 ± 58.9 mm Hg, respectively. The differences of the SFP between the healthy and each of the diseased groups were statistically significant (P < 0.01 to 0.05). Meanwhile, SFP of nine patients with cerebral thrombosis and 18 patients with hemorrhage was measured during their time course of the diseases from the onset to 180 days. SFP in the acute stage of thrombosis showed an increase and a gradual decrease during the time course, while SFP in the acute stage of hemorrhage showed the opposite—a decrease and a gradual increase. A statistically significant difference was observed between both groups within 30 days from the onset (P < 0.01). Screen filtration pressure in the acute stage of hemorrhage showed 95.2 ± 17.7 mm Hg in nine survival cases and 194.0 ± 96.2 mm Hg in nine deaths within ten days from the onset. The difference was statistically significant (P < 0.01). Such results suggest a role of platelets in cerebral thrombosis and hemorrhage and a usefulness in differential diagnosis of both diseases.

Introduction

RECENTLY we have reported hyperaggregability of platelets by adenosine 5'-diphosphate (ADP) and epinephrine in the acute stage of thromboembolic disorders including cerebral thrombosis. In the above report, the platelet aggregability was measured by the optical density (OD) method used by Born* and O'Brien.† Though the OD method is the most popular method for detecting platelet aggregability, it was not possible to avoid destruction or injury of platelets during the centrifugation procedure required for preparation of platelet-rich plasma as a testing sample. The screen filtration pressure (SFP) method was originated by Swank,* who used whole blood to detect platelet aggregation. Thus, injury to the platelets during centrifugation, which could affect platelet sensitivity to ADP, can be avoided. Therefore, we measured platelet aggregability in patients with cerebral thrombosis or hemorrhage using the SFP method and compared the above values to those of patients with essential hypertension and of healthy subjects of the same age.

Methods

Three hundred eighty-three patients, including 50 healthy volunteers, were utilized. They were divided into four groups, i.e., healthy, essential hypertensives with more than 160 mm Hg systolic and more than 95 mm Hg diastolic blood pressure, and those with cerebral thrombosis or hemorrhage in their recovery stage with onset occurring more than two months before the examination. There were 25 men and 25 women, age 52 to 78 years (average and SD: 64.0 ± 9.4), as the healthy volunteers; 43 men and 50 women, age 43 to 85 years (65.6 ± 8.5), had essential hypertension; 111 men and 55 women, age 41 to 84 years (62.4 ± 11.8), were in the recovery stage of cerebral thrombosis; and 50 men and 24 women, age 36 to 84 years (57.4 ± 10.0), were in the recovery stage of cerebral hemorrhage. The distribution of age in each of these groups was almost identical. In addition, three men and six women, age 49 to 84 years (66.8 ± 9.5), with cerebral thrombosis and ten men and eight women, age 52 to 87 years (66.4 ± 10.6), with cerebral hemorrhage were examined from the onset to 180 days after the stroke. Four patients with cerebral hemorrhage also were able to be examined prior to the stroke. Cerebral thrombosis and hemorrhage were diagnosed by using the definition cited by Millikan et al. One patient with cerebral thrombosis and nine with cerebral hemorrhage died while in the acute stage. These diagnoses were confirmed by autopsy. The patients were not given anticoagulants or drugs which might affect platelet function, such as aspirin, dipyridamole and pyridinolcarbamate during the observation period.

To estimate platelet aggregability by the SFP method, 9 ml of blood were collected from the cubital vein of the subjects using a plastic disposable syringe containing 1 ml of

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3.8% sodium citrate solution. Stasis was not applied. Within one hour after collection, the platelets in the citrated whole blood were examined for their ability to be aggregated by ADP using the SFP method. For this purpose we used an SFP apparatus (Chapman Precision Products, Inc., Bangor, Maine). Blood (2.7 ml) was added into a plastic tube containing 0.3 ml of ADP solution in a concentration of 30 μM. Thus the final concentration of ADP solution in the blood was 3 μM. The blood was stirred by a magnetic stirrer at a constant speed for 60 seconds and 2.4 ml of blood were collected in a plastic syringe to transfer to the SFP apparatus. Sixty seconds after mixing the blood and ADP, the syringe piston was pushed by a mechanically driven ram to obtain SFP for ten seconds. The blood was forced at a steady rate through a screen with multiple micropores, 20 × 20 micra square and approximately 20 micra deep. The resistance to flow of blood (in mm Hg) through the screen was measured by a transducer. The pressure curve was designated SFP curve and the final pressure at the end of ten seconds was described as the SFP in mm Hg. Usually, SFP was measured twice in the same sample, and the average was obtained as an SFP of the subject. Using the same sample, plasma fibrinogen levels were measured by a weight method. The results were described as averages and SD, and compared using Student’s t-test between each of the groups.

Chemicals
Disodium salt of ADP (Sigma Chemical Co., St. Louis, Missouri) was used for the test. Stock ADP was deep-frozen at a concentration of 10⁻⁴M, and the pH of the solution was adjusted at 7.4 with Tris-buffer. The working solution of ADP for the test was prepared by dilution with Tris-buffer to obtain a concentration of 30 μM.

Results
The SFP values, which were estimated twice on the same sample, were similar and within the 95% confidence limits. SFP of the healthy elderly people was in the range of 43 to 299 and its average and SD were 148.7 ± 53.5 mm Hg. In these subjects, SFP of the men was 142.9 ± 47.9 mm Hg, and SFP of the women was 154.5 ± 62.3 mm Hg. There was no statistically significant difference in the SFP between the men and women. Also, no statistically significant correlation was found between the SFP and the distribution of age as shown in table 1. SFP of the patients with hypertension and cerebral hemorrhage and thrombosis in the recovery stage was 176.2 ± 74.4, 189.8 ± 58.3 and 206.3 ± 58.9 mm Hg, respectively. SFP of the hypertensive, cerebral hemorrhagic and thrombotic patients had higher values than that of the healthy subjects (P < 0.01 to 0.05) (fig. 1). However, there was no significant difference between those with thrombosis. Simultaneously, the plasma fibrinogen level of the same sample was measured. Plasma fibrinogen levels of the healthy, hypertensive and recovery stage of hemorrhagic and thrombotic patients were 277.7 ± 54.8, 338.4 ± 103.7, 340.9 ± 95.3 and 353.8 ± 94.4 mg/dl, respectively. A statistically significant difference was observed between the fibrinogen level of the healthy subjects and each of the diseased groups (P < 0.05). However, there was no correlation between the SFP and the fibrinogen level.

On the contrary, SFP in the acute stage of cerebral hemorrhage and thrombosis showed different changes. SFP of four patients, before onset of cerebral hemorrhage, showed 126, 150, 186 and 256 mm Hg respectively. Their SFP showed 88, 135, 148 and 198 mm Hg one to two days after the onset of stroke. Including another 14 cases, SFP of 18 patients with cerebral hemorrhage showed a decrease shortly after the onset of stroke and an increase gradually during their time course (fig. 2). In the above cases, nine patients died within ten days from the onset. Their SFP showed 194.0 ± 96.2 mm Hg after the stroke. The SFP of the nine survival cases showed 95.2 ± 17.7 mm Hg within ten days from the onset. There was a statistically significant

Table 1 Age and Sex Differences on Platelet Aggregability

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Men</th>
<th>Women</th>
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</thead>
<tbody>
<tr>
<td>50-59</td>
<td>165.7 ± 57.8</td>
<td>151.9 ± 60.4</td>
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<tr>
<td>60-69</td>
<td>136.3 ± 42.5</td>
<td>109.5 ± 60.1</td>
</tr>
<tr>
<td>70-79</td>
<td>126.7 ± 36.6</td>
<td>102.5 ± 55.7</td>
</tr>
<tr>
<td>Total</td>
<td>142.9 ± 47.0</td>
<td>154.5 ± 62.3</td>
</tr>
</tbody>
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Platelet Aggregability in Healthy
Hypertension
Cerebral Hemorrhage
and Thrombosis

Figure 1. Platelet aggregability (SFP by 3 μM ADP) in hypertensive patients and those with cerebral hemorrhage and thrombosis in the recovery stage was significantly higher than that of the healthy group (P < 0.01 to 0.05).
difference between both groups (P < 0.01). Meanwhile, SFP of nine patients with cerebral thrombosis showed an increase shortly after the onset and a decrease gradually during the time course of the disease (fig. 2). When SFP in the acute stage was measured following the time course of the diseases, SFP of 18 patients with hemorrhage showed 142.4 ± 52.5 and SFP of nine patients with thrombosis showed 241.3 ± 65.4 mm Hg within ten days after the onset. SFP of 11 patients with hemorrhage showed 182.6 ± 60.5 and SFP of nine patients with thrombosis showed 282.1 ± 69.9 mm Hg after 11 to 30 days from the onset. Within 30 days from the onset, SFP of cerebral hemorrhage showed a lower value than that of thrombosis, with a statistical significance of P < 0.01 (fig. 3). After 31 to 180 days from the onset, SFP of nine patients with hemorrhage showed 182.6 ± 60.5, and SFP of 21 patients with thrombosis showed 235.5 ± 47.7 mm Hg; there was no statistically significant difference between both of these values. These values were similar to those measured in the other large number of cases in the recovery stage of stroke.

Discussion

We found hyperaggregability of platelets by ADP in the patients with essential hypertension and those with cerebral hemorrhage and thrombosis in the recovery stage when they were compared to that of the age-matched healthy people using the SFP method. A marked increase of aggregation within 30 days from the onset was observed especially in those patients with cerebral thrombosis. Such phenomenon also was found in the acute stage of thromboembolic disorders including cerebral thrombosis using the OD method. In our observation, a correlation was observed between the SFP value and the intensity of secondary aggregation using the OD method (P < 0.05), but the correlation coefficient was not as high, e.g., 0.534. This suggests that both methods show a platelet aggregability, but both aggregabilities represent behavior of platelets from a different standpoint. The SFP method has an advantage whereby it avoids destruction of or injury to platelets during the centrifugation procedure as compared to the OD method. We could not find hyper-aggregability of platelets in the recovery stage of cerebral thrombosis using the OD method. It may show a different character of the SFP method from the OD method. As the SFP method used whole blood, it is possible that several factors in the blood may influence SFP. However, Dhall and Matheson found no significant correlation between SFP and platelet count when the platelet count was between 15
and $35 \times 10^3/\mu l$. They also found that variation in the hematocrit between 35% and 45% did not produce significant changes of the SFP. In our previous observation, there was no close relationship between SFP and RBC, hematocrit or platelet count in clinical cases. As described in the present results, plasma fibrinogen level also did not show any correlation with SFP, although the fibrinogen level of the diseased groups was higher than that of the healthy subjects. In our observation, we could not find fibrin strands in the used screen of the SFP method by scanning electron microscopic findings. In the used screen, only typical figures of platelet aggregation induced by ADP were seen. Dhall and Mathesoon found that the accuracy within the 95% confidence limits of a single observation by SFP was approximately 20%. When the mean result of three observations was taken, the accuracy within similar limits was approximately 10%. In our study each of the SFP values, which were estimated twice for each sample, showed approximately the same value within the 95% confidence limits.

When the SFPs of the patients with cerebral thrombosis and hemorrhage in the recovery stage, of those with essential hypertension, and of the healthy people were compared, the highest SFP was found in the thrombotic group, decreasing approximately 20%. When the mean result of three observations was taken, the accuracy within similar limits was approximately 10%. In our study each of the SFP values, which were estimated twice for each sample, showed approximately the same value within the 95% confidence limits.

Meanwhile we found no correlation between age and SFP in the elderly persons. In younger people, women had higher SFPs than men and an effect of estrogen on platelet aggregability was suggested. However, for older persons with menopause, the sex difference in SFP was not observed.

In the acute stage of cerebral hemorrhage, SFP showed a marked decrease. It is very interesting that SFP in thrombosis and hemorrhage in the acute stage showed an opposite change. In our observation, the decrease was observed in subarachnoid bleeding also. We could not yet determine whether these changes were cause or effect of the diseases. All four cases in which we could measure SFP before and after the onset of cerebral hemorrhage showed decreases of SFP of 25 to 100 mm Hg shortly after the stroke. In general, cerebral hemorrhage may occur without abnormalities in the blood coagulation system and platelet function. So the hyperaggregability of platelets observed may be due to an effect of cerebral hemorrhage. In cerebral thrombosis, SFP at 11 to 30 days after the stroke was higher than within ten days after the onset. It may suggest that an effect of thrombosis induces a hyperaggregability of platelets. In a previous report, we have pointed out the importance of interaction of injured inner surface of blood vessels and platelet aggregability. In relation to this phenomenon, it is interesting that simultaneous appearance of hyper-aggregability of platelets, decrease of adhesive platelet count and increase of blood coagulability occurred immediately after an exercise test on coronary sclerotic patients.

In the present report, we have observed a difference in SFP in the cerebral hemorrhage group between those patients who survived and those who died. The survivors showed higher SFP within ten days from the onset than those who died. It may be due to the grade of hemorrhage in the brain or to an effect of stress due to fatal cerebral damage on a living body. It has been reported that platelet aggregability is affected by some kind of stress such as a mental stress, an injection of epinephrine, etc. The characteristic changes of platelet aggregability observed by SFP method may reflect pathophysiological changes between cerebral thrombosis and hemorrhage and may be useful for a differential diagnosis of both diseases.

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