Inhibitory Effect of Acetylsalicylic Acid on Platelet Function in Patients With Completed Stroke or Reversible Ischemic Neurologic Deficit

Ti-Kai Lee, MD, Yao-Chang Chen, MD, I-Nan Lien, MD, Mei-Ching Liu, MD, and Zei-Shung Huang, MD

The purpose of our study was to investigate the effects of different doses of acetylsalicylic acid on platelet aggregation. Among inpatients of the National Taiwan University Hospital, 236 cases of completed stroke and seven cases of reversible ischemic neurologic deficit that were diagnosed by computed tomography of the brain and that had not ingested acetylsalicylic acid or acetylsalicylic acid-like drugs for >2 weeks before admission were selected for this study. Thromboxane B₂ and 6-keto-PGF₁α were measured by radioimmunoassay, threshold concentration of adenosine diphosphate was measured by Born’s method, and circulating platelet aggregates were measured by the method of Wu and Hoak. Various single doses of acetylsalicylic acid (75, 300, or 600 mg) or 300 mg acetylsalicylic acid every 6 hours for four doses or one dose of 300 mg acetylsalicylic acid with 75 mg dipyridamole significantly suppressed the mean plasma thromboxane B₂ concentrations and elevated the mean adenosine diphosphate threshold concentrations. Abnormal plasma thromboxane B₂ concentrations, adenosine diphosphate threshold concentrations, or circulating platelet aggregate ratios were significantly normalized after administration of these regimens. The effects were not significantly different among treatment groups. Forty milligrams of acetylsalicylic acid seemed to have less platelet-inhibitory effect. A single dose of 75 mg acetylsalicylic acid significantly inhibited platelet hyperfunction and effectively corrected the abnormal plasma thromboxane B₂ concentrations, adenosine diphosphate threshold concentrations, and circulating platelet aggregate ratios. Higher doses did not enhance the inhibitory effect. In addition, this single dose of acetylsalicylic acid did not significantly suppress plasma 6-keto-PGF₁α. We conclude that 75 mg acetylsalicylic acid per day is adequate to inhibit platelet hyperfunction. (Stroke 1988; 19:566–570)

It has been documented that acetylsalicylic acid (ASA) has an inhibitory effect on platelet function through specific blocking of the enzyme cyclooxygenase; thus, this antiplatelet agent is now widely used for the prevention of ischemic cerebrovascular disease.

Treatment with oral administration of 1,000–1,300 mg ASA/day has been reported to have a significantly beneficial effect in male patients with threatened stroke, in patients with a history of multiple transient ischemic attacks (TIAs), and in the secondary prevention of atherothrombotic cerebral infarction or to have no favorable influence in patients with reversible cerebral ischemic attacks. However, an increasing number of studies have documented that high doses of ASA might depress not only thromboxane A₂ (TXA₂) but also prostacyclin (PGI₁). Since PGI₁ has an inhibitory effect on platelet activity, suppression of this prostaglandin could possibly have adverse effects on ischemic stroke patients. Consequently, the optimal antithrombotic dose of ASA has recently been the subject of much controversy.

Our study was conducted to investigate the effects of various doses of ASA on platelet aggregation and prostaglandins in patients with completed stroke or reversible ischemic neurologic deficit (RIND) and to search for the optimal dose of this drug for inhibition of platelet hyperactivity.

Subjects and Methods

Inpatients of the National Taiwan University Hospital included 236 cases of completed stroke and seven cases of RIND that had not ingested ASA or ASA-like drugs for >2 weeks before admission and in which the absence of serum salicylate (SA) had been confirmed participated in this study. Besides the clinical criteria, computed tomograms (CT scans) of the brain were employed in all cases for making a diagnosis. A hypodense lesion, location of the lesion, edema, mass effect, density changes of the lesion in serial CT scans, and contrast enhancement of the lesion were the important elements for diagnosis of an infarction. Patients with a large lesion including involvement of the entire vascular territory of a major vessel (anterior, middle, or posterior cerebral artery) were not selected. Evidence of an infarction was found on a CT scan of
the brain in 237 of the 243 cases, 229 in the carotid and eight in the vertebrobasilar territory. No recognizable lesions were found on serial CT scans of the brain or by other examinations in the remaining six cases. Diagnoses were made according to clinical criteria in these six cases; two were considered carotid territory infarctions and four vertebrobasilar infarctions. Those that had auricular fibrillation or possible cardiac sources of emboli were not included in this study.

The ages of the 149 men in our study ranged from 45 to 86 years, with a mean ± SD of 63.1 ± 8.6 years. The ages of the 94 women ranged from 45 to 86 years, with a mean ± SD of 63.5 ± 9.4 years.

Various doses of ASA were given in tablet form to 235 patients from 3 to 10 days after the onset of stroke and to eight patients from 11 to 21 days after the onset. There were still some neurologic deficits at the time of study in all patients. Those who had severe neurologic deficits, disturbances of consciousness, or difficulty in swallowing were not included. The severity of ischemic damage was not significantly different among the groups taking various doses of ASA.

After an overnight fast, blood samples for the determination of serum SA levels, platelet aggregation, and prostaglandin concentrations were collected using a 19-gauge butterfly needle for venipuncture, and the first few drops of blood were discarded. It has been reported that in vivo platelet inhibition of prostaglandin synthesis is virtually complete by 60–120 minutes after administration of ASA. Therefore, blood was drawn before and 2 hours after ingestion of a different single dose of ASA (40, 75, 300, or 600 mg) or one dose of 300 mg ASA in combination with 75 mg dipyridamole (DP). Those patients who ingested 1,200 mg ASA took 300 mg every 6 hours starting at noon, with the fourth dose administered at 6:00 AM the next morning. Blood was then drawn at 8:00 AM.

Serum SA concentrations were measured using Trinder's method. TXB₂ and 6-keto-PGF₁α (the stable products of TXA₂ and PGI₂, respectively) were measured using a radioimmunoassay kit (New England Nuclear, Boston, Massachusetts). The blood was drawn into polypropylene tubes containing one-tenth volume of 3.8% sodium citrate with 1 mM acetylsalicylic acid to prevent prostaglandin and TXB₂ formation after sampling. Then the blood was centrifuged at 1,000g for 20 minutes at 4°C to prepare the plasma for determinations of TXB₂ and 6-keto-PGF₁α. All patients had a normal platelet count (200–350 x 10⁹/l) and hematocrit (35–45%). The determinations were performed in duplicate with appropriate standards.

For evaluation of in vitro platelet aggregation, the turbimetric method of Born was employed using a Bio-Data aggregometer (Hatboro, Pennsylvania), and the lowest concentration of adenosine diphosphate (ADP) inducing a full biphasic response was recorded as the threshold concentration. A lowered threshold concentration of ADP indicates hyperaggregability of platelets.

For evaluation of in vivo platelet aggregation, circulating platelet aggregates (CPAs) were detected by the ethylenediaminetetraacetic acid (EDTA)-formalin method of Wu and Hoak. The CPA ratio was then calculated as CPA ratio = platelet count in EDTA-formalin + platelet count in EDTA. A low CPA ratio indicates stronger platelet aggregability.

In our series the mean ± SD normal values were plasma TXB₂ concentration 29.54 ± 13.68 pg/ml (n = 45), ADP threshold concentration 4.79 ± 1.54 μM (n = 35), and CPA ratio 0.89 ± 0.09 (n = 45). A plasma TXB₂ concentration greater than mean + 2SD was considered to be abnormally high, and an ADP threshold concentration or a CPA ratio of less than mean – 2SD was considered to be abnormally low.

For the determination of significance, a paired t test was used for the differences between pre- and post-ASA values, and McNemar’s test was used for the difference in correcting effects on abnormal values, that is, binary variables. As the SDs were large, raw data were logarithmically transformed in the comparison of group means using repeated-measurement analysis of variance.

Results
After ingestion of a single dose of 40 mg ASA, the mean plasma TXB₂ concentration was significantly depressed (p < 0.02), but the mean ADP threshold concentration was not affected (Table 1). After administration of the five other regimens, mean plasma TXB₂ concentrations were significantly depressed (Table 1).

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**Table 1. Influence of Various Doses of Acetylsalicylic Acid Alone and With Dipyridamole on Plasma TXB₂ Concentrations and ADP Threshold Concentrations**

<table>
<thead>
<tr>
<th>Acetylsalicylic acid (mg)</th>
<th>Dipyridamole (mg)</th>
<th>No. cases</th>
<th>Plasma TXB₂ concentrations (pg/ml)</th>
<th>ADP threshold concentrations (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before medication</td>
<td>After medication</td>
<td>p</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>14</td>
<td>83.14 ± 45.76</td>
<td>39.74 ± 37.01</td>
</tr>
<tr>
<td>75</td>
<td>0</td>
<td>56</td>
<td>94.48 ± 81.92</td>
<td>22.96 ± 30.86</td>
</tr>
<tr>
<td>300</td>
<td>0</td>
<td>51</td>
<td>90.96 ± 78.87</td>
<td>17.80 ± 33.29</td>
</tr>
<tr>
<td>600</td>
<td>0</td>
<td>31</td>
<td>96.10 ± 57.11</td>
<td>15.07 ± 21.30</td>
</tr>
<tr>
<td>1,200</td>
<td>0</td>
<td>33</td>
<td>88.97 ± 52.45</td>
<td>13.31 ± 18.65</td>
</tr>
<tr>
<td>300</td>
<td>75</td>
<td>20</td>
<td>88.15 ± 56.44</td>
<td>17.14 ± 23.67</td>
</tr>
</tbody>
</table>

Values are mean ± SD. p by paired t test; NS, not significant.
Plasma TXB₂ concentration in normal persons, 29.54 ± 13.68 pg/ml (n = 45); >56.9 pg/ml was considered abnormally high.
ADP threshold concentration in normal persons, 4.79 ± 1.54 μM (n = 35).
The mean ADP threshold concentrations were within normal limits but increased significantly after medication in all groups (Table 1). The effects on mean plasma TXB₂ concentrations and ADP threshold concentrations were not significantly different among the five groups.

The normalizing effects of the six regimens on abnormal plasma TXB₂ concentrations, ADP threshold concentrations, and circulating platelet aggregate ratios are shown in Table 2.

Abnormally high TXB₂ concentrations returned to normal after medication in 7 of 10 patients in the 40 mg ASA group, 29 of 34 in the 75 mg ASA group, 28 of 33 in the 300 mg ASA group, 20 of 22 in the 600 mg ASA group, 11 of 12 in the 1,200 mg ASA group, and 6 of 7 in the ASA + DP group. There was one patient each in the 40 mg ASA and the 75 mg ASA groups whose normal ADP threshold concentrations became abnormal after ingestion of the drug. Statistically, except for the 40 mg ASA group, ADP threshold concentrations in the other five groups were significantly restored to normal (Table 2).

Abnormally low CPA ratios were corrected to normal in 3 of 3 patients in the 40 mg ASA group, 21 of 26 in the 75 mg ASA group, 22 of 27 in the 300 mg ASA group, 10 of 12 in the 600 mg ASA group, 10 of 11 in the 1,200 mg ASA group, and 6 of 7 in the ASA + DP group. There was one patient each in the 40 mg ASA and the 75 mg ASA groups whose normal CPA ratios became abnormal after ingestion of the drug. Statistical analysis revealed that the correcting effect on abnormal CPA ratios was not significant in the 40 mg ASA group but was significant in the other five groups (Table 2). It is probable that the results in the 40 mg ASA group were due to the small number of patients.

We also observed the effects of various doses of ASA on 6-keto-PGF₁α. Since 6-keto-PGF₁α concentrations of <10 pg/ml were undetectable by the method we used, only patients having higher 6-keto-PGF₁α concentra-
The optimal ASA dose for the prevention of ischemic stroke is still currently the subject of much controversy. In our study, administration of a single dose of 40 mg ASA significantly depressed the mean concentration and normalized abnormal concentrations of plasma TXB, but did not affect the mean ADP threshold concentrations, nor did it normalize abnormal ADP threshold concentrations. Abnormally low CPA ratios were normalized in all three patients by 40 mg ASA, yet the effect was not significant. Although there is a possibility that these results were due to the small sample size, the platelet-inhibitory effect of this dose seemed weaker than that of higher doses. It has been reported that 50 mg ASA/day produced a substantial depression of TXB levels, but this dose was insufficient to inhibit aggregation.

However, after ingestion of a single dose of 75, 300, or 600 mg ASA or 300 mg ASA every 6 hours four times, mean plasma TXB concentrations decreased and mean ADP threshold concentrations increased significantly. These four doses of ASA also had significant correcting effects on abnormal plasma TXB concentrations, ADP threshold concentrations, and CPA ratios. The effects were not significantly different among the four ASA doses. This indicates that an increase in the dose of ASA did not augment the effects. Furthermore, 75 mg ASA did not depress plasma 6-keto-PGF\textsubscript{1a}. From these experimental results we postulate that a single dose of 75 mg ASA is adequate for inhibition of platelet hyperfunction.

Arterial thrombosis and a number of other diseases have been attributed to an imbalance in the PGI\textsubscript{1}-TXA\textsubscript{2} system.\textsuperscript{8} The chief purpose of ASA treatment in ischemic stroke is to correct this imbalance by inhibiting platelet cyclooxygenase, which converts arachidonic acid to TXA\textsubscript{2}. But the use of higher doses of this drug will inhibit vascular wall cyclooxygenase concomitantly, and thus reduce PGI\textsubscript{1}.\textsuperscript{5-8,10} TXA\textsubscript{2} induces platelet aggregation and PGI\textsubscript{1} inhibits aggregation; therefore, many investigators have tried to find the optimal dose of ASA that will inhibit formation of TXA\textsubscript{2} without affecting PGI\textsubscript{1} production.

It has been reported that the doses of ASA that reduced TXA\textsubscript{2} synthesis but had no effect on PGI\textsubscript{1} synthesis were 40 mg/day,\textsuperscript{4} 50 mg/day,\textsuperscript{14} 1-2 mg/kg,\textsuperscript{16} 0.5 mg/kg,\textsuperscript{12} or 0.45 mg/kg.\textsuperscript{11} A dose of 300 mg ASA was reported to inhibit PGI\textsubscript{1} generation in normal veins.\textsuperscript{7} In our experiment, 300 mg ASA significantly inhibited plasma 6-keto-PGF\textsubscript{1a}.

Recently, the results of many studies have been in favor of the use of low-dose ASA. Fifty to seventy milligrams ASA per day satisfactorily inhibited platelet aggregation and prolonged bleeding time in patients with recurrent cerebrovascular events.\textsuperscript{15} A dose of 3.1 mg/kg of this drug suppressed the development of thrombus, but when the dose was increased the antithrombotic effect disappeared.\textsuperscript{13}

On the contrary, some evidence indicated that a higher dose of ASA might be needed. ASA was found to have two effects on hemostasis. One was achieved with a low dose of ASA and the other with a much higher dose. It is probable that high-dose ASA could concurrently inhibit synthesis of TXA\textsubscript{2} and 12-hydroxyeicosatetraenoic acid, which is important in preventing platelet deaggregation. Besides, ASA has limitations as an antithrombotic drug. In some cases of cerebral infarction, there might be rapid formation and sudden massive release of aggregation inducers such as thrombin, ADP, and collagen. ASA was unable to suppress platelet activation induced by high doses of such agents.\textsuperscript{21}

In our study, the use of 300 mg ASA with 75 mg DP, a phosphodiesterase inhibitor, did not show a stronger antiplatelet effect than 75, 300, 600, or 1,200 mg ASA alone. The American-Canadian Co-Operative Study Group\textsuperscript{23} reported that the addition of DP contributed nothing in TIA patients taking ASA. But in another report, DP alone showed a modest antithrombotic effect augmented by a low but not a high dose of ASA.\textsuperscript{10}

In summary, our study showed that 75 mg ASA significantly depressed the mean plasma TXB concentration and elevated the mean ADP threshold concentration and effectively corrected abnormal plasma TXB concentrations, ADP threshold concentrations, and CPA ratios. Higher doses did not enhance this effect. Administration of 75 mg ASA did not significantly inhibit plasma 6-keto-PGF\textsubscript{1a}, but ingestion of 300 mg ASA did. Forty milligrams ASA showed less platelet inhibitory effect. Therefore, we conclude that 75 mg ASA is adequate for suppression of platelet hyperactivity.

**Acknowledgments**

The authors would like to thank professor Ching-Chang Hung for revising the language of the text and Misses Andrey Ang, Li-Li Kuo, Hsiao-Hwa Lin, Chi-Jen Wan, and Mei-Hwa Chen for their technical assistance.

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Key Words • aspirin • cerebral ischemia • cerebrovascular disorders • platelets
Inhibitory effect of acetylsalicylic acid on platelet function in patients with completed stroke or reversible ischemic neurologic deficit.

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*Stroke.* 1988;19:566-570
doi: 10.1161/01.STR.19.5.566

*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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/19/5/566

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