Effects of Low-to-High Doses of Aspirin on Platelet Aggregability and Metabolites of Thromboxane A₂ and Prostacyclin

Hideo Tohgi, MD; Shu Konno, MD; Kenichi Tamura, MD; Bunsho Kimura, MD; and Katsumi Kawano, PhD

Background and Purpose: The purpose of this study was to compare the effects of low-to-high doses of aspirin on platelet aggregability determined by different methods and on the metabolism of thromboxane A₂ and prostacyclin.

Methods: We administered increasing doses (40, 320, and 1,280 mg/day) of aspirin to 19 poststroke patients and studied the differences in 1) the changes in platelet aggregability depending on the methods of evaluation and 2) the concentrations of prostaglandin metabolites in the blood and urine.

Results: Aggregation of platelet-rich plasma induced by a strong stimulus (10 μM ADP) was significantly reduced after 40 mg/day aspirin (p<0.005), and this reduction was similar to that after higher aspirin doses. In contrast, aggregation of platelet-rich plasma induced by weaker stimuli (1 and 5 μM ADP) decreased less significantly after 40 mg/day aspirin compared with that after higher aspirin doses. The serum thromboxane B₂ generated after ex vivo incubation was reduced significantly (by 85%) after 40 mg/day aspirin and decreased further after 320 mg/day (by 96%) and 1,280 mg/day (by >99%) of aspirin. The urinary 11-dehydro-thromboxane B₂ concentration decreased less significantly after 40 mg/day aspirin (by 42%) compared with that after 320 mg/day (by 78%) and 1,280 mg/day (by 91%) aspirin doses. The urinary concentration of 2, 3-dinor-6-keto-prostaglandin F₁α did not decrease after 40 mg/day aspirin but decreased significantly after higher doses of aspirin.

Conclusions: These findings suggest that different doses of aspirin may be necessary to prevent thrombogenesis induced by different triggers of different strengths and that 40 mg/day aspirin is able to inhibit a large proportion of maximum thromboxane A₂ release provoked acutely, with the prostaglandin I₂ synthesis being little affected; however, higher doses of aspirin are required to attain further inhibition.

KEY WORDS • aspirin • platelet aggregation • thromboxane B₂

Aspirin, a potent antiplatelet agent, is widely used for prevention of atherothrombotic diseases. It exerts antiplatelet action by inhibiting cyclooxygenase, thus reducing the synthesis of thromboxenic thromboxane (TX) A₂ in platelets. However, it also inhibits cyclooxygenase in vascular endothelial cells and reduces their production of prostaglandin (PG) I₂, which has an antithrombotic action. It has been demonstrated that at low doses (less than 100 mg/day), the reduction in TXB₂ (the metabolite of TXA₂) was remarkable, and the production of 6-keto-PGF₁α (the metabolite of PGI₂) was little affected. Clinical trials also have shown that 30 mg/day aspirin was as effective as 283 mg/day aspirin for the prevention of vascular events² and that 75 mg/day aspirin significantly reduced the risk of stroke or death compared with a placebo.³ However, there are still some problems that need further study. First, the amount of reduction in platelet aggregability required to prevent atherothrombotic disease has not yet been established. This is largely because the degree of platelet aggregation inhibition differs depending on the method of determination (e.g., whole-blood impedance or light transmission) and the aggregating agents and their concentrations. Second, recent studies have indicated that 11-dehydro-TXB₂ (a metabolite of TXB₂)⁴⁻⁷ and 2,3-dinor-6-keto-PGF₁α (a metabolite of 6-keto-PGF₁α)⁸ are more stable and reliable indicators than TXB₂ and 6-keto-PGF₁α, respectively. Few systematic data, however, are available concerning the effects of aspirin doses on these metabolites.

We therefore administered low-to-high doses of aspirin to poststroke patients in the chronic phase and compared platelet aggregability measured by different methods, induced by different aggregating agents and concentrations; we also compared the concentrations of serum TXB₂, urinary 11-dehydro-TXB₂, and urinary 2,3-dinor-PGF₁α.
Subjects and Methods

We studied 19 patients (7 men and 12 women, 71.9±6.3 years) in the chronic phase of cerebral thrombosis. Nine were hypertensive, eight had diabetes, and four were smokers. Since we did not find a significant influence of the baselines on their results, we combined these patients for analysis. None of the patients had taken nonsteroidal anti-inflammatory medication for more than 1 month before the study. The study period was 7 weeks; the patients received 40 mg/day aspirin for the first week and did not receive aspirin for the second and third weeks (washout period); they then received 320 mg/day aspirin for the fourth week, followed by a washout period during the fifth and sixth weeks; and they received 1,280 mg/day for the seventh week. We determined all the parameters described below before treatment and on the last days of the first, fourth, and seventh weeks of aspirin administration for each dose. Informed consent was obtained from all patients.

For platelet aggregation studies, venous blood was taken by venipuncture and put into tubes containing sodium citrate for platelet aggregation. Aggregation in both platelet-rich plasma (PRP) and whole blood were studied. The PRP aggregation was measured by percent maximum change in light transmission using a Born aggregometer (Niko Bioscience, Tokyo). We also inspected aggregation curves and determined whether the primary aggregation only or both primary and secondary aggregation occurred. Aggregating reagents used were 2 μg/ml collagen (Chrono-Log, Havertown, Pa.) and 1, 5, and 10 μM adenosine diphosphate (ADP; Sigma Chemical Co., St. Louis, Mo.). Whole-blood platelet aggregation was estimated using a Chrono-Log Model 540 whole-blood aggregometer (Coulter Electronics Ltd., Luton, UK); aggregating reagents were 2 μg/ml collagen. Rate of aggregation was assessed by the change in impedance (ohms) 6 minutes after adding the reagents. The linear relation between changes in impedance at 6 minutes and the maximum change have been confirmed.

For determination of serum TXB2, nonanticoagulated blood was allowed to clot at 37°C for 90 minutes to permit maximal generation of TXA2 by platelets in response to endogenously produced thrombin. The separated sera were kept at −20°C until assayed. The TXA2 production was studied indirectly by measuring the concentration of TXB2 in serum. Urine was collected for 24 hours.

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TABLE 1. Platelet Aggregation and Prostaglandin Concentrations After Different Doses of Aspirin Compared With Premedication

<table>
<thead>
<tr>
<th>Light transmission (%)</th>
<th>Before aspirin</th>
<th>40 mg/day</th>
<th>320 mg/day</th>
<th>1,280 mg/day</th>
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<tr>
<td>1 μM ADP</td>
<td>46.5±23.7</td>
<td>45.6±16.4</td>
<td>28.8±10.1†</td>
<td>28.8±9.1†</td>
</tr>
<tr>
<td>5 μM ADP</td>
<td>72.1±12.3</td>
<td>64.9±5.8*</td>
<td>59.2±6.9†*</td>
<td>54.5±9.2†*</td>
</tr>
<tr>
<td>10 μM ADP</td>
<td>76.2±11.5</td>
<td>66.4±5.5*</td>
<td>64.7±6.9†*</td>
<td>65.5±8.4†*</td>
</tr>
<tr>
<td>2 μg/ml collagen</td>
<td>73.4±8.6</td>
<td>59.2±16.3†</td>
<td>44.9±17.7††</td>
<td>43.7±16.7††</td>
</tr>
</tbody>
</table>

Whole-blood aggregation (ohm)

| Collagen (2 μg/ml)     | 23.2±3.4      | 23.8±5.0   | 14.8±4.3†‡ | 13.1±3.3†‡  |
| Serum TXB2 (ng/ml)    | 48.12±40.40   | 7.13±10.31†| 1.77±0.28† | 0.08±0.14†§|
| Urinary 11-dehydro-TXB2 (pg/ml) | 994±620  | 515±351†  | 217±174†§  | 90±61†§    |
| Urinary 2,3-dinor-6-keto-PGF1α (pg/ml) | 213±96   | 196±59    | 108±59†§  | 74±51†§    |

Values are mean±SD. n, Number of patients; ADP, adenosine diphosphate; TX, thromboxane; PG, prostaglandin.

* (p<0.05), † (p<0.005) compared with pretreatment values.
§ (p<0.05), ‡ (p<0.005) compared with values after 40 mg/day aspirin treatment.
|| (p<0.05) compared with values after 320 mg/day aspirin treatment.

The significant decreases in the 10-μM ADP–induced aggregation of platelet-rich plasma after 40 mg/day aspirin as well as after higher doses suggest that the inhibition of an irreversible platelet aggregation induced by strong stimuli appears to be maximum with low-dose aspirin. On the other hand, we found that aggregation of platelet-rich plasma induced by weaker stimuli (1 and 5 μM ADP) could not be inhibited or was less inhibited by 40 mg/day aspirin compared with higher doses. These apparently paradoxical findings may be explained by the fact that 1 μM ADP induces mainly the primary aggregation and that aspirin essentially inhibits the secondary aggregation. Our findings based on inspection of the aggregation curves may support this assumption. Our results also demonstrated that 40 mg/day aspirin is insufficient to attenuate whole-blood aggregation induced by 2 μg/ml collagen (i.e., approximately 3–4 μg/ml in plasma, if we consider the hematocrit), suggesting that a low dose of aspirin appears to be less effective for inhibition of platelet aggregation when both red and white blood cells are present than in the solution consisting exclusively of...

TABLE 2. Number of Patients with Aggregation Curves Indicating Primary Aggregation Alone or Both Primary and Secondary Aggregation

<table>
<thead>
<tr>
<th>Aspirin dosage</th>
</tr>
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<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>40 mg</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>1 μM ADP</th>
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<tbody>
<tr>
<td>Primary</td>
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<tr>
<td>Primary and secondary</td>
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<th>5 μM ADP</th>
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n, Number of patients; ADP, adenosine diphosphate.

*p<0.005, †p<0.05 compared with pretreatment values by χ² test.
platelets. However, further studies are needed on whole-blood aggregation induced by higher concentrations of collagen to evaluate this assumption.

The remarkable (85%) reduction in serum TXB₂ generation in vitro after 40 mg/day aspirin, which is consistent with previous reports, indicates that the inhibition of a large proportion of the maximum capacity of acutely induced TXB₂ release from platelets is achieved by low-dose aspirin. Although we do not know whether the rate of reduction is sufficient to inhibit thrombogenesis in vivo, our results for urinary 2,3-dinor-6-keto-PGF₁α indicate that PG₁α synthesis in endothelial cells is not inhibited after 40 mg/day aspirin, which is consistent with the conclusion derived from the determination of the 6-keto-PGF₁α concentration.

Thus, our findings may provide the basis for the reported effects of low-dose aspirin for stroke prevention. However, because there is an individual variation in the platelet inhibition with low-dose aspirin, moderately higher doses of aspirin may be required for relatively low responders. In such cases, 10-μM ADP-induced aggregation of platelet-rich plasma and serum TXB₂ determinations may be appropriate indices for monitoring the effects of aspirin.

The observed dose-dependent decrease of urinary 11-dehydro-TXB₂ with the increasing doses of aspirin suggests that TXA₂ secretion of platelets in baseline or ordinary conditions is inhibited less significantly with 40 mg/day aspirin compared with higher aspirin doses. The maximum capacity of platelets for TXA₂ production that still remains uninhibited with 40 mg/day aspirin (15% of the pretreatment value) but is inhibited only by higher doses of aspirin may have an important significance for baseline TXA₂ secretion as reflected in urinary 11-dehydro-TXB₂. Because the important role of platelets in the pathogenesis of atherosclerosis has been established, such a sustained level of TXA₂ may contribute to further progression of atherosclerosis. It has been reported that the plasma 11-dehydro-TXB₂ level is a useful parameter to discriminate between stroke patients and controls.

In conclusion, for average subjects 40 mg/day aspirin is able to significantly reduce the maximum capacity of TXA₂ formation of platelets and aggregation induced by strong stimuli, without the inhibition of PG₁α production. However, the reduction in baseline TXA₂ production of platelets, aggregation induced by weak stimuli, and whole-blood aggregation were achieved only with higher aspirin doses, the clinical significance of which needs further scrutiny.

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References

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