Individual Variation in Platelet Aggregability and Serum Thromboxane B₂ Concentrations After Low-Dose Aspirin

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The effects of low daily oral doses of aspirin (40 mg/day) on platelet aggregability and serum thromboxane B₂ concentrations were studied in 19 poststroke patients. Although platelet aggregation was reduced significantly after 1 week, there was wide individual variation in the inhibition of platelet function in spite of marked decreases of serum thromboxane B₂ concentrations by >90% (from 224 ± 58 to 8 ± 8 ng/ml). There was no correlation between collagen-induced platelet aggregability and serum thromboxane B₂ concentration before aspirin administration in the range 100-350 ng/ml, but after 1 week of repeated administration of aspirin, there was a correlation between platelet aggregability and serum thromboxane B₂ concentrations of <25 ng/ml (r=0.68, p<0.01). However, platelet inhibition was insufficient even in some patients with markedly decreased thromboxane B₂ concentrations (<5 ng/ml). Our results suggest that individual variation of platelet aggregability in response to low-dose aspirin may be due to variation not only in the degree of inhibition of thromboxane A₂ production but also in the relative dependence of platelet aggregation on extra-arachidonic pathways. (Stroke 1988;19:700–703)

Aspirin, now widely used as an antiplatelet agent, inhibits the synthesis of thromboxane A₂ (TXA₂) in platelets by irreversibly acetylating the active site of cyclooxygenase while simultaneously inhibiting the production of prostacyclin (PGI₁) in blood vessels. To avoid this “aspirin dilemma,” low doses of aspirin are now widely recommended based on the assumption that a low dose can inhibit thromboxane synthesis in platelets with much less effect on PGI₁ production in vascular endothelium.

Low-dose aspirin (1 mg/kg or 50–70 mg/day) has been shown to prolong bleeding time and ADP-induced platelet aggregation.¹ A larger dose of 150 mg aspirin has been reported to completely inhibit the epinephrine-induced release reaction of platelets in vitro.² Patrignani et al³ showed that a daily dose of 0.45 mg/kg aspirin produced a cumulative and virtually complete inhibition of platelet TXB₂ production, while the urinary excretion of prostaglandins FGE, PGF, and 6-keto-PGF₁α and the furosemide-induced renal synthesis of PGI₁ were not affected significantly. After single doses of 80 mg aspirin, the generation of TXB₂ in serum was reported to be reduced more than PGI₁ production, relative to control in aorta and saphenous vein tissues removed at surgery.⁴

While it is widely recognized that the inhibitory effect of aspirin on platelet function varies among individuals, it is not known whether this variability depends on the degree of inhibition of thromboxane synthesis in platelets.

Our study was designed to assess platelet aggregation and its correlation with concentrations of serum TXB₂, a stable metabolite of TXA₂, after daily administration of low doses of aspirin (40 mg/day).

Subjects and Methods

Nineteen poststroke patients were studied (9 men and 10 women, aged 48–82 years). None of these patients had taken nonsteroidal anti-inflammatory drugs for >1 month before the study. Aspirin (40 mg/day) was given to the patients for 4 weeks. Platelet aggregation and serum TXB₂ were measured before initial ingestion of aspirin and after 1 and 4 weeks of daily administration of aspirin.

Platelet Aggregation

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. PRP aggregation was measured by percent maximum change in light transmission using a Born aggregometer (Niko Bioscience, Tokyo, Japan); aggregating reagents used were 2 μg/ml collagen, 10 and 1 μM ADP (Sigma Chemical Co., St. Louis, Missouri) and 10 μM epinephrine. Whole-blood platelet aggregation was estimated using a Chronolog Model 540 whole blood aggregometer (Coulter Electronics Ltd., Luton, U.K.); aggregating reagents were 2 μg/ml collagen, 10 μM ADP, and 100 μM epinephrine. 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.
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Collagen (2 μg/ml) ADP (10 μM) ADP (1 μM) Epinephrine (10 μM)

Weeks of repeated oral administration of aspirin (40 mg/day)

FIGURE 1. Changes of platelet aggregability in platelet-rich plasma measured by light transmission method before and after 1 and 4 weeks of administration of 40 mg/day aspirin. *Significantly inhibited, p<0.01.

Serum Thromboxane B₂ Determinations

Nonanticoagulated blood was allowed to clot at 37°C for 30 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. TXA₂ production was studied indirectly by measuring the concentration of TXB₂ in serum by radioimmunoassay using specific antibodies and tritiated tracers (New England Nuclear Corp., Boston, Massachusetts).

Results

Using the light transmission method (Figure 1), platelet aggregation in PRP was suppressed to almost the same degree after 1 and 4 weeks of daily administration of aspirin. Compared with pretreatment values platelet aggregation after 4 weeks averaged 47% for collagen, 78% for ADP, and 50% for epinephrine. There was, however, wide individual variation in the degree of platelet inhibition, as exemplified in Figure 2 in which collagen-induced platelet aggregability is plotted for each patient before and after administration of aspirin.

With the impedance method (Figure 3), collagen-induced aggregation in whole blood was significantly inhibited to 66% after 1 week and to 34% after 4 weeks of daily aspirin compared with pretreatment values. Epinephrine-induced aggregation was significantly inhibited to 53% after 1 week and to 39% after 4 weeks. ADP-induced aggregation did not change significantly.

Mean ± SD TXB₂ concentration in PRP was 224 ± 58 ng/ml before aspirin and decreased remarkably to 8 ± 8 ng/ml (3.6% of the pretreatment value, p<0.01) after 1 week and to 26 ± 41 ng/ml (11.6% of pretreatment value, p<0.01) after 4 weeks of repeated daily administration of aspirin.

Collagen-induced platelet aggregability in PRP before aspirin administration was not correlated with serum TXB₂ concentration in the range 100–350 ng/ml (Figure 4), but after 1 week of daily aspirin, there was a correlation between collagen-induced platelet aggregability and serum TXB₂ concentrations of <25 ng/ml (r=0.68, p<0.01; Figure 5). There are two groups of patients, those with TXB₂ concentrations of 20–25 ng/ml and maximum transmission of >60% and those with TXB₂ concentrations of <12 ng/ml and maximum transmission ranging from 10% to 60%. In some of the latter patients, platelet aggregability did not decrease remarkably in spite of marked reduction of TXB₂ levels.

Whole-blood aggregation measured by the impedance method was not significantly correlated with serum TXB₂ levels after administration of aspirin.

Discussion

It has been reported that a single oral dose of aspirin below a ceiling of 2 mg/kg produces a dose-dependent reduction of serum TXB₂ concentration; above a dose of 2 mg/kg, a ceiling effect (>95%) is reached. In a study of inactivation of platelet cyclooxygenase, a ceiling effect was reached after only a single 650-mg dose. Our study demonstrates a significant decrease (of >90%) of serum TXB₂ after 40 mg/day aspirin. Although platelet aggregation induced by collagen, ADP, and epinephrine in both PRP and whole blood...
was also significantly suppressed, there was nevertheless wide individual variation.

Although the magnitude of platelet inhibition required to prevent cerebrovascular stroke is unknown, 40 mg/day aspirin appears to be ineffective in patients whose platelet aggregability remains within the range mean of pretreatment values ± SD after administration of aspirin. Our results suggest that there are at least two categories of patients in whom platelet inhibition is insufficient with low doses of aspirin, those with insufficient reduction of TXB₂ production (20–25 ng/ml), and those with insufficient inhibition of platelet function in spite of remarkable reduction of TXB₂ production (<5 ng/ml).

Results in the first group of patients suggest that platelet aggregation is impaired only after almost-complete inhibition of thromboxane production. In the second group it is not surprising that only mild inhibition of platelet function was attained despite virtually complete block of TXB₂ synthesis since other patients who have congenital deficiency of cyclooxygenase and thromboxane synthetase activity demonstrate only a mild hemostatic defect similar to that induced by aspirin.⁷ These facts suggest the importance of other factors such as extra-arachidonate pathways⁶ in some individuals.

Previous reports have indicated a cumulative inhibitory effect of aspirin during the first week of treatment, but no further cumulative effect has been observed thereafter.¹² Our study showed that platelet inhibition reached a maximum 1 week after the initiation of aspirin administration when platelet function was measured by the light transmission method using PRP but that whole-blood aggregation measured by the impedance method was more inhibited 4 weeks than 1 week after the initiation of aspirin. Our findings suggest a delayed or cumulative indirect effect of aspirin on platelet aggregation through its effects on erythrocytes and/or leukocytes.

In conclusion, low doses of aspirin (40 mg/day) are ineffective for suppressing platelet function in some individuals; platelet inhibition may be attained by increasing daily doses of aspirin in some individuals but not in others.

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References


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