Timing of Anti-Platelet Effect After Oral Aspirin Administration in Patients With Sympathetic Excitement

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Aspirin is used in percutaneous coronary interventions (PCI) for acute myocardial infarction (AMI) to prevent thrombosis. It is reported that the aspirin concentration in blood reaches its peak approximately 20 min after oral administration in healthy volunteers, but the absorption and bioavailability of aspirin in AMI may be quite different. In the present study patients undergoing coronary angiogram for the first time were enrolled as a model of sympathetic excitement and the timing of the antiplatelet effect after oral aspirin administration was investigated. Aspirin (162 mg) was administered to the patients in a catheter laboratory. Platelet count, aspirin concentration, and platelet aggregation were measured at scheduled timepoints before and up to 120 min. Ticlopidine was administered in the same procedure, and platelet count and platelet aggregation were evaluated at 0 and 120 min. There was no significant change in the platelet count. Aspirin concentration in blood had not reached its peak by 120 min. Platelet aggregation induced by collagen or ADP began to be inhibited 45 min after aspirin administration. No significant inhibition of platelet aggregation was observed up to 120 min following ticlopidine administration. During sympathetic excitement, aspirin absorption and its antiplatelet effect were significantly delayed in these patients. Ticlopidine did not show any antiplatelet effect by 120 min. For PCI performed in a patient with a high level of sympathetic excitement, aspirin should be administered at least 45 min before the first balloon dilatation. (Circ J 2003; 67: 697–700)

Key Words: Antiplatelet effect; Aspirin; Percutaneous coronary intervention; Sympathetic excitement

Methods

Patients

Twenty male patients who were suspected of having ischemic heart disease and who underwent a coronary angiogram examination for the first time were enrolled in this study. Aspirin was administered to one group (n=10) and ticlopidine to the other (n=10). Informed consent was obtained from all patients. None of them had received any medication for at least 10 days before the study.

Drugs

Aspirin tablets (buffered aspirin tablet, 81 mg) and ticlopidine (100 mg) were purchased from Lion (Tokyo, Japan) and Daiichi Pharmaceutical, Co (Tokyo, Japan), respectively. For the purpose of simulating the treatment of AMI, buffered aspirin was chosen, because its absorption was considered to be much faster than that of enteric-coated aspirin.

First Study Protocol (Aspirin Group)

Two tablets of aspirin (=162 mg) were administered with 100 ml of water immediately prior to the coronary angiogram examination in the catheter laboratory. The patients had fasted for at least 12 h. Blood samples were collected 0, 15, 30, 45, 60, and 120 min after aspirin administration. Ticlopidine was administered in the same procedure, and platelet count and platelet aggregation were evaluated at 0 and 120 min. There was no significant change in the platelet count. Aspirin concentration in blood had not reached its peak by 120 min. Platelet aggregation induced by collagen or ADP began to be inhibited 45 min after aspirin administration. No significant inhibition of platelet aggregation was observed up to 120 min following ticlopidine administration. During sympathetic excitement, aspirin absorption and its antiplatelet effect were significantly delayed in these patients. Ticlopidine did not show any antiplatelet effect by 120 min. For PCI performed in a patient with a high level of sympathetic excitement, aspirin should be administered at least 45 min before the first balloon dilatation.

Platelet Count

Platelets were counted electronically with an automatic blood cell counter.

Plasma Aspirin Concentration

To determine the plasma concentration of aspirin, 10 mg NaF and 1 mg EDTA·2 Na were added to blood samples. The plasma was separated by centrifugation (3,000 rpm, 5 min.) and then stored at −20°C until analysis by Biochemical and Pharmacological Labs, Inc (Osaka, Japan) according to the method previously described. In brief, the aspirin and internal standard (methyl p-hydroxybenzoate) were separated from the plasma by adding HCl and extracting the compounds into chloroform. The organic fraction was evaporated to dryness, and then separated...
using high-performance liquid chromatography with a pre-packed stainless-steel column (4.0×250 mm I.D, Develosil Ph, Nomura Chemical) and a mobile phase (methanol: 10 mmol/L phosphoric buffer (pH 2.6) = 1:3) at 0.8 ml/min. The temperature was kept at 35°C. Quantitation of aspirin was achieved by ultraviolet detection at 237 nm. All chemicals were of analytical grade or better.

**Platelet Aggregation**

Platelet aggregation was measured by the turbidometric method described by Born. In brief, platelet-rich plasma was prepared from 4.5 ml of blood with 0.5 ml of 3.8% sodium citrate by centrifugation at 150 g for 10 min. Platelet aggregation was performed in siliconized cuvettes by stirring at 1,000 rpm in an NKK platelet aggregation tracer. Aggregation was induced by collagen at 1 and 5 μg/ml, and adenosine diphosphate (ADP) at a concentration of 2 μmol/L.

**Second Study Protocol (Ticlopidine Group)**

One tablet of ticlopidine (100 mg) was administered using the same procedure as in protocol 1. Blood samples were collected at 0, and 120 min after taking ticlopidine. Platelet aggregation was measured in the same manner.

**Statistical Analysis**

Data were expressed as mean±SD. Statistical analysis was performed with unpaired Student’s t test. A value of p<0.05 was considered statistically significant.

**Results**

The clinical characteristics of the patients in both the aspirin and ticlopidine groups are depicted in Table 1. Hepatic and renal functions were all in the normal range, and the left ventricular function evaluated by echocardiography was normal. A deviated high blood concentration of cholesterol or triglyceride, which might affect the platelet aggregation, was not observed. Thus, it was considered worthwhile assessing pharmacokinetics in these groups.

Fig 1 shows the time course of the platelet counts following aspirin administration. Neither giving 162 mg of aspirin nor a coronary angiographic examination altered the platelet count.

The plasma aspirin concentration following 162 mg of aspirin administration is shown in Fig 2. Plasma aspirin was detected 15 min after administration, and the concentration had time-dependently increased. The peak of the serum aspirin concentration had still not been observed 120 min after aspirin administration.

Collagen-induced platelet aggregation at a concentration of 1 μg/ml was 76.7±5.0% at 0 min (= just before aspirin administration). The aggregation was significantly inhibited to 33.7±16.5% by 45 min. With 5 μg/ml of collagen, platelet aggregation at 0 min was 80.7±2.3%, and started to be significantly inhibited to 51±7.0% by 45 min (Fig 3). Although similar results were obtained for ADP-induced aggregation, the inhibitory effect was less prominent than with collagen-induced aggregation (Fig 4).

Fig 5 demonstrates the effect of ticlopidine on the platelet count and collagen-induced platelet aggregation at concentrations of 1 and 5 μg/ml. Neither alteration of the platelet count nor inhibitory effects against platelet aggregation was observed up to 120 min after ticlopidine administration.

**Discussion**

In this study, we enrolled patients undergoing a coronary angiogram for the first time because we considered they would have a high level of sympathetic excitement at the prospect of undergoing an invasive examination, surrounded by intimidating medical equipment, and being observed by a number of medical staff. Under these conditions, the absorption and bio-availability of drugs and the status of platelets could well differ from normal circumstances. Moreover, the present study model is consid-
erated to be closer to actual clinical conditions, because it was conducted with patients lying down and with both middle-aged and elderly subjects (50–82 years) suspected of suffering from ischemic heart disease, and undergoing coronary angiography examination.

It is reported that among healthy young volunteers (aged 19–38 years), the peak blood aspirin concentration after oral administration is reached in approximately 20 min. In the present study, the increase in blood aspirin concentration was delayed compared with those previous reports, and the peak was not observed by 120 min after administration. The study also demonstrated that the anti-platelet effect of aspirin occurred 45 min following oral administration. In the present study we used 2 different concentrations of collagen (1 and 5 µg/ml) and 2 µmol/L of ADP for measuring platelet aggregation. Although a significant inhibitory effect of aspirin against platelet aggregation was observed, such an effect was more prominent in collagen-induced aggregation at a concentration of 1 µg/ml. These results suggest that prior to PCI for AMI, aspirin should be admin-

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**Fig. 3.** Collagen-induced platelet aggregation following aspirin administration. (A) Representative platelet aggregation curve induced by 1 µg/ml of collagen. (B) Summary of platelet aggregation induced by 1 and 5 µg/ml of collagen. Data are mean ± SD. (n=10). *p<0.05, **p<0.01 (compared with 0 min).

**Fig. 4.** ADP-induced platelet aggregation following aspirin administration. (A) Representative platelet aggregation curve induced by 2 µmol/L of ADP. (B) Summary of platelet aggregation induced by 2 µmol/L of ADP. Data are mean ± SD. (n=10). *p<0.05, **p<0.01 (compared with 0 min).
istered at least 45 min before the first balloon dilatation.

It was recently reported that 30–40% of the population are aspirin non-responders; however, none was observed in our present study. This discrepancy might be related to differences in the concentration of platelet aggregation agonists. Kawasaki et al tested 0.375–0.75 μg of collagen and concluded that a lower collagen concentration is more likely to detect an aspirin non-responder whereas we used 1 and 5 μg of collagen. A further study using lower collagen concentrations and higher doses of aspirin might be useful.

In the present study, ticlopidine, one of the more commonly used anti-platelet drugs, still had not shown an inhibitory effect on platelet aggregation 120 min after administration. This result was compatible with previous findings that aspirin non-responders; however, none was observed in our present study. This discrepancy might be related to differences in the concentration of platelet aggregation agonists. Kawasaki et al tested 0.375–0.75 μg of collagen and concluded that a lower collagen concentration is more likely to detect an aspirin non-responder whereas we used 1 and 5 μg of collagen. A further study using lower collagen concentrations and higher doses of aspirin might be useful.

Study Limitations
The first weakness of this study is that the patients did not have chest symptoms on examination, whereas AMI patients ordinarily have severe chest pains and may vomit. This suggests that the absorption and bioavailability of aspirin might be delayed even more in patients with an actual AMI. Further study with such patients may clarify the practical dynamism of the drug. A second weakness is that the present test was performed with fasting patients. The timing of the prior meal and the amount of food ingested may affect drug absorption. It might not be easy to address this study limitation because a patient’s stomach condition varies with the clinical situation.

Conclusion
Among patients experiencing sympathetic excitement, aspirin absorption is delayed and may take at least 45 min before its anti-platelet effect occurs. Ticlopidine did not have an anti-platelet effect even 120 min after administration. We conclude from this that aspirin should be administered soon enough after diagnosis of AMI to allow at least 45 min before the first balloon procedure in coronary revascularization therapy.

References