Increased Rate of Formation of Small-Sized Platelet Aggregates in Patients With Acute Coronary Syndromes

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Coronary thrombosis has been implicated in the pathogenesis of acute coronary syndromes, and platelet activation plays a pivotal role in the pathogenesis of coronary thrombus. A new platelet aggregometer using a laser-light scattering beam was trialled for assessment of platelet aggregation. Platelet aggregability, especially small-sized platelet aggregates, was investigated on admission using the laser-light scattering method and again after treatment in 23 patients with acute coronary syndromes. The platelet aggregability in 14 patients with stable exertional angina and in 14 control subjects was also examined. On admission, the number of small- and medium-sized platelet aggregates in the acute coronary syndromes group was significantly greater than in the stable exertional angina group or control group. However, the number of large-sized platelet aggregates on admission was not increased in the acute coronary syndromes group. Furthermore, the number of small- and medium-sized platelet aggregates decreased significantly after treatment in the acute coronary syndromes group. The increased number of small-sized platelet aggregates may sensitively reflect attacks of thrombosis in patients suffering acute coronary syndromes. (*Jpn Circ J* 2000; 64: 647–652)

Key Words: Acute coronary syndromes; Laser-light scattering method; Platelet aggregability

Coronary thrombosis plays an important role in the pathogenesis of acute coronary syndromes, including unstable angina, acute myocardial infarction and ischemic sudden death.^{1–3} We and other investigators^{4–8} have reported that the plasma level of fibrinopeptide A, a sensitive marker of thrombin generation, and the activity of plasma plasminogen activator inhibitor^{9,10} an indicator of the impairment of fibrinolysis, increase in patients with unstable angina and acute myocardial infarction.¹¹

Platelet activation therefore plays an important role in the pathogenesis of acute coronary syndromes.^{12–14} Platelet aggregation, conventionally measured using either the optical density method¹⁵ or impedance method¹⁶ is indispensable for the clinical evaluation of platelet function. A recently developed platelet aggregability measuring system using a laser-light scattering method^{17,18} is capable of monitoring the increase in the size of small aggregates (9–25 μ m, ie, ≤ 100 platelets), which can not be detected with the conventional methods. It has been demonstrated that the formation of medium- and large-sized platelet aggregates begins with small-sized platelet aggregates,^{17,18} so the production of small-sized platelet aggregates is very important in initial thrombus formation. Using the new aggregometer, Eto et al have shown that the number of small aggregates increased in acute coronary syndromes.¹⁹

In the present study, also using the new laser-light scatter-

ing method, we measured platelet aggregability, both on admission and after treatment, in patients with acute coronary syndromes (acute myocardial infarction and unstable angina pectoris) and those with stable exertional angina, as well as control subjects. We compared the results with data from the conventional optical density method.

Methods

Study Population

Fifty-one patients (35 men, 16 women; mean age, 65 ± 1 years, mean $\pm$ SEM, range 40–84), who underwent diagnostic cardiac catheterization, were studied. They were divided into an acute coronary syndromes group, a stable exertional angina group, and a control group. The acute coronary syndromes group consisted of 23 patients (10 acute myocardial infarction and 13 unstable angina pectoris; 16 men, 7 women; mean age 66 ± 2 years) who had attacks of chest pain associated with ST segment elevation or depression on ECG. The 10 patients with acute myocardial infarction (7 men, 3 women) were admitted within 12 h of symptom onset and the diagnosis was made on the basis of chest pain persisting for ≥ 30 min, ST-segment elevation of >0.2 mV in more than 2 contiguous leads on a standard 12-lead ECG, and elevation of the serum creatine kinase-MB isoenzyme level to more than twice the upper limit of normal. The average time from the onset of symptoms to admission was 7.4 ± 1.8 (mean $\pm$ SEM) h (range 1–12). The 13 patients with unstable angina pectoris comprised 9 men and 4 women and their condition was defined as a crescendo pattern of chest pain at rest with documented transient ST-segment depression or ST-segment elevation of at least 0.1 mV in at least 2 contiguous ECG leads. Their last spontaneous attack

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was required to have occurred within 1 h of entry into the study. There were no patients with ST-segment changes lasting more than 30 min, with new Q waves, or with raised plasma concentrations of creatine kinase or creatine kinase-MB. The stable exertional angina group consisted of 14 patients (11 men, 3 women; mean age 68 ± 2 years) who had typical exertional angina and $\geq 90\%$ narrowing of the major coronary artery. The most recent attack in each patient of this group had occurred at least 4 weeks before entry into the study. No changes in the frequency of angina, precipitating factors, or responses to sublingual nitroglycerin were noted in the previous 3 months in this study group. The control group consisted of 14 patients (8 men, 6 women; mean age 59 ± 2 years) who had atypical chest pain unaccompanied by ECG changes and without significant coronary arterial stenosis ($\leq 25\%$ of luminal diameter), or coronary spasm, as demonstrated by an intracoronary injection of acetylcholine.

The stable exertional angina and control groups were selected from patients undergoing elective cardiac catheterization during the same period as the acute coronary syndromes group and were matched for age, gender and other clinical variables.

In the patients with acute coronary syndromes, oral medications were usually started on the first day of admission and were not discontinued at the time of coronary arteriography. In the patients with stable exertional angina, oral medications were usually administered in hospital. The study patients were not treated with antiplatelet drugs other than aspirin. Factors suspected of reflecting platelet aggregation, such as the hematocrit, platelet count and total cholesterol, were not significantly different between the 3 groups (Table 1). Patients taking coumarin anticoagulant were excluded from this study. The study protocol was approved by the Ethics Committee and written informed consent was obtained from each patient and his or her family.

Coronary Arteriography

Emergency coronary arteriography was performed on admission in 6 patients with acute myocardial infarction. In 4 patients with acute myocardial infarction and 13 with unstable angina pectoris, medical treatment were prescribed and coronary arteriography was performed 2–4 weeks after admission. In the patients with stable exertional angina and the control subjects, coronary arteriography was performed by the Judkins technique using contrast material in the morning when the patients were fasting.

After heart rate and blood pressure measurements, control angiography of the left main and right coronary arteries was performed. The patients with acute coronary syndromes who did not have significant organic stenosis and the control subjects received an intracoronary acetylcholine injection to provoke coronary spasm.²⁰ Thereafter, isosorbide dinitrate was administered to detect organic coronary lesions.

Blood Sampling

Blood samples for measuring the platelet count, hematocrit and lipid concentration were drawn using a 21-gauge needle inserted into an antecubital vein while the subject lay recumbent; 4.5 ml of blood was collected into tubes containing sodium citrate. Venous blood samples were obtained on admission before the start of heparin or other drugs in patients with acute coronary syndromes. Because it is considered that platelet aggregability increases signifi-

cantly during the morning,²¹ repeat venous blood samples from the 23 acute coronary syndrome patients were taken at 07.00 h in the fasting state 2–4 weeks after admission. Blood samples from the stable exertional angina and control subjects were obtained at 07.00 h in the fasting state after admission. The sample was left for 15 min at room temperature, then centrifuged at 150 G for 10 min at room temperature in order to obtain platelet-rich plasma. The remaining sample was then centrifuged at 300 G for 10 min at room temperature in order to obtain platelet-poor plasma.

Platelet Aggregometer

Platelet aggregability was assessed using the aggregometer (PA-200, Kowa, Tokyo, Japan) recently developed by Ozaki et al.^{17,18} the principles of which have been described previously. Briefly, a laser beam measuring $40 \mu\text{m}$ in diameter was generated using a 20 mW diode laser (675 nm, Toshiba, Tokyo, Japan) and passed through platelet-poor plasma ($300 \mu\text{l}$) stirred in a 5-mm internal diameter cylindrical glass cuvette. The light scattered from the observation volume ($48 \times 140 \times 20 \mu\text{m}$) was detected by a photocell array, with light intensity corresponding to particle size. The signal frequency was recorded at 10-s intervals. Data were expressed as the change over time (s) in the number of aggregates (counts/s) of individual sizes (determined by light intensity, expressed in volts). Particles with an intensity of 25–400 mV represented small-sized platelet aggregates ($9\text{--}25 \mu\text{m}$), those with an intensity of 400–1,000 mV represented medium-sized platelet aggregates ($25\text{--}50 \mu\text{m}$), and those with an intensity of 1,000–2,048 mV represented large-sized platelet aggregates ($50\text{--}70 \mu\text{m}$). Changes in total intensity were recorded at 10-s intervals for 5 min. Platelet aggregation was assessed in a spontaneous state and in the presence of ADP (0.5, 1.0, and $3.0 \mu\text{mol/L}$; $1.0 \mu\text{mol/L}$ of ADP was used as the aggregating reagent because a preliminary study had determined that detection of small-sized platelet aggregation was most sensitive at this concentration). We compared the results with data from the conventional optical density method.

Effect of Aspirin

Aspirin therapy is of proven value in the treatment of acute myocardial infarction as well as long-term in patients with a wide range of prior manifestations of cardiovascular disease.²² To examine whether aspirin had an influence on platelet aggregation, we measured platelet aggregates before and after 81 mg/day of aspirin treatment in 12 patients with stable exertional angina as a preliminary study.

Statistical Analysis

All data are expressed as mean $\pm$ SEM. The comparisons of continuous data among the 3 groups (acute coronary syndromes group, stable exertional angina group, and control group) were analyzed by a one-way analysis of variance. When these results were statistically significant, they were analyzed by Fisher's test. Changes in platelet aggregation in patients with acute coronary syndromes on admission and after treatment were analyzed by paired t test. The frequency data were compared by a chi-square test. Probability levels < 0.05 were considered significant.

Results

Patients' Characteristics and Clinical Outcomes

The clinical characteristics of the study population are

Table 1 Clinical Characteristics of the Study Groups

Characteristics	Acute coronary syndrome (n=23)	Stable exertional angina (n=14)	Controls (n=14)
Age (years)			
Mean	66±2	68±2	59±3
Range	40–84	50–77	46–81
Gender (n) M/F	16/7	11/3	8/6
Hematocrit (%)	36.5±1.3	36.7±1.0	38.0±1.6
Platelet count (×10 ⁴ /mm)	19.3±1.1	19.6±2.0	18.5±1.7
Coronary risk factors			
Hypertension (BP ≥140/90 mmHg, n)	16	8	9
Diabetes mellitus (n)	13	5	2
Obesity (BMI ≥25, n)	7	4	5
Smoking (n)	14	8	3
Hyperlipidemia (mg/dl)			
Serum total-cholesterol	173±6	179±13	193±14
Serum LDL-cholesterol	99±7	106±11	123±10
Serum HDL-cholesterol	43±3	49±6	56±4
Serum triglyceride	131±39	119±22	138±19
Extent of coronary vessel organic stenosis ≥75% stenosis (n)			
0-vessel	3	0	14
1-vessel	10	9	0
2-vessel	3	2	0
3-vessel	7	3	0
Drug			
Aspirin	7	10	0
Calcium antagonists	6	10	0
Long-acting nitrates	5	6	0
β-blockers	0	6	0
Angiotensin-converting enzyme inhibitors	2	4	0

Values are mean ± SEM. BMI, body mass index; BP, blood pressure.

Table 2 Treatment of Patients With Acute Coronary Syndromes

Age (years) / Gender	Diagnosis	Treatment	Culprit lesion	Medication before admission
63/M	AMI	Direct PTCA	RCA	None
80/F	AMI	Direct PTCA	LCx	None
46/F	AMI	Direct PTCA	LAD	None
79/F	AMI	Direct PTCA	LAD	None
72/M	AMI	Direct PTCA	LAD	None
40/M	AMI	ICT+rescue PTCA	RCA	None
84/M	AMI	Medication	LAD	None
70/M	AMI	Medication	LAD	None
83/M	AMI	Medication	LCx	None
70/M	AMI	Medication	LCx	None
69/M	UAP	Medication	LCx	None
52/M	UAP	Medication	RCA	Aspirin, calcium antagonist, nitrate
72/M	UAP	Medication	LAD (Coronary spasm)	Calcium antagonist
57/F	UAP	Medication	LAD (Coronary spasm)	Calcium antagonist
57/M	UAP	Medication	LCx	Aspirin, nitrate
69/M	UAP	Medication	LCx (Coronary spasm)	None
51/M	UAP	Medication	LAD	Aspirin, nitrate, ACE inhibitor
64/M	UAP	Medication	LCx	Aspirin, calcium antagonist, nitrate
73/F	UAP	Medication	LAD	None
71/F	UAP	Medication	LAD	Aspirin, calcium antagonist
52/M	UAP	Medication	LCx	Aspirin, calcium antagonist
71/F	UAP	Medication	LAD	None
68/M	UAP	Medication	LAD	Aspirin, nitrate, ACE inhibitor

PTCA, percutaneous transluminal coronary angioplasty; ICT, intracoronary thrombolysis; AMI, acute myocardial infarction; UAP, unstable angina pectoris; RCA, right coronary artery; LAD, left anterior descending artery; LCx, left circumflex artery; ACE, angiotensin-converting enzyme.

detailed in Table 1. There were no significant differences among the 3 groups in terms of age, sex, hypertension, smoking, diabetes mellitus, obesity, serum total cholesterol, serum high density lipoprotein (HDL) cholesterol, serum low density lipoprotein (LDL) cholesterol or serum triglyc-

eride levels. The medications of the patients with acute coronary syndromes are detailed in Table 2. Of the patients with acute myocardial infarction, 6 of 10 had emergency coronary angiography, and 5 of 6 patients underwent direct percutaneous transluminal coronary angioplasty (PTCA) (3

Fig 1. Comparison of the number of platelet aggregates using the laser-light scattering method and the optical density method. (A) Small-sized platelet aggregates, (B) medium-sized platelet aggregates, (C) large-sized platelet aggregates, (D) optical density method.

Table 3 Comparison Among the 3 Groups

Platelet aggregates	Acute coronary syndromes		Stable exertional angina	Controls
	On admission	After treatment		
Small-sized (V)	$6.1 \pm 1.1 \times 10^4$ ***††	$2.2 \pm 0.8 \times 10^4$ *	$3.5 \pm 0.8 \times 10^3$	$2.6 \pm 0.8 \times 10^3$
Medium-sized (V)	$1.5 \pm 0.6 \times 10^4$ **†	$1.4 \pm 0.4 \times 10^3$	$8.6 \pm 1.0 \times 10^2$	$1.0 \pm 0.2 \times 10^3$
Large-sized (V)	$3.8 \pm 0.9 \times 10^3$	$2.9 \pm 0.5 \times 10^3$	$2.7 \pm 0.5 \times 10^3$	$1.6 \pm 0.5 \times 10^3$
Optical density method (%)	16.0 ± 2.1 ††	8.7 ± 1.1	7.5 ± 1.5	6.3 ± 1.0

Values are mean $\pm$ SEM.

*** $p < 0.0001$ vs stable exertional angina and control subjects, ** $p < 0.01$ vs stable exertional angina and control subjects, * $p < 0.05$ vs stable exertional angina and control subjects, †† $p < 0.0001$ vs after treatment, † $p < 0.01$ vs after treatment, † $p < 0.05$ vs after treatment.

in left anterior descending artery, 1 in right coronary artery, and 1 in left circumflex artery). One of the 6 patients had intracoronary thrombolysis, after which rescue PTCA was performed. PTCA was successful in all patients (residual stenosis $\leq 50\%$) and no abrupt closure of the infarct-related coronary artery occurred. In the remaining 4 patients with

acute myocardial infarction, medical treatment was prescribed and coronary arteriography was performed 2–4 weeks after admission.

All the patients with unstable angina pectoris received medical treatment on admission: increasing doses or the addition of β -blockers in 4 patients, calcium antagonists in

Table 4 Comparison of Platelet Aggregates Before and After 81 mg of Aspirin Treatment in Patients With Stable Exertional Angina

Platelet aggregates	Before aspirin	After aspirin	p value
Small-sized (V)	$8.9 \pm 3.3 \times 10^3$	$5.8 \pm 1.6 \times 10^3$	NS
Medium-sized (V)	$1.4 \pm 0.3 \times 10^3$	$9.5 \pm 1.6 \times 10^2$	NS
Large-sized (V)	$2.7 \pm 0.3 \times 10^3$	$1.7 \pm 0.4 \times 10^3$	<0.05
Optical density method (%)	9.6 ± 1.3	5.9 ± 0.8	<0.05

Values are mean $\pm$ SEM.

2 patients, long-acting nitrates in 6 patients, and aspirin in 12 patients. In all 13 patients with unstable angina pectoris, coronary arteriography was performed 2–4 weeks after admission.

Twenty patients with acute coronary syndromes had significant organic stenosis of 90% or more of the diameter in the coronary arteries. Three patients with unstable angina pectoris and no organic coronary stenosis were documented to have had coronary spasms. All the patients with stable exertional angina had major coronary arterial stenosis of $\geq 90\%$ of the diameter. The control subjects had no significant coronary stenosis and no coronary spasms ($\leq 25\%$ diameter).

Platelet Aggregation

The number of small-sized platelet aggregates on admission was significantly higher in the acute coronary syndromes group than in the stable exertional angina group or the control group ($p < 0.0001$) (Fig 1A; Table 3). Similarly for medium-sized ($p < 0.05$), but not the large-sized platelet aggregates (Fig 1B,C; Table 3). Platelet aggregations on admission were significantly higher in the acute coronary syndromes group than in the stable exertional angina group or control group using the optical density method ($p < 0.01$) (Fig 1D; Table 3).

Platelet aggregates were reexamined after medical treatment in the 23 patients with acute coronary syndromes. The number of small-sized platelet aggregates after treatment decreased significantly ($p < 0.0001$), but was still significantly higher than that in the stable exertional angina group or control group ($p < 0.05$) (Fig 1A; Table 3). The number of medium-sized platelet aggregates after treatment also decreased significantly to the levels observed in the stable exertional angina group and control group ($p < 0.05$) (Fig 1B; Table 3). However, the number of large-sized platelet aggregates after treatment did not change significantly (Fig 1C; Table 3). Platelet aggregations measured with the optical density method decreased significantly after treatment in the acute coronary syndromes group ($p < 0.01$) (Fig 1D; Table 3).

Twenty-two of the 23 patients in the acute coronary syndromes group were administered 81 mg/day of aspirin after hospitalization, as did 12 other patients with stable exertional angina as a preliminary study in which it was found that aspirin suppressed production of large-sized platelet aggregates, but had no influence on that of small or medium-sized platelet aggregates (Table 4). These patients were not included in the present study. At baseline, the number of small-sized platelet aggregates was slightly higher in the patients with stable exertional angina in the preliminary study than in those of the present study, which may be due to the difference of the frequency of complicated coronary risk factors between the 2 groups, in particular the proportion of patients with the complication of diabetes mellitus were higher in the preliminary study (8 of 12

patients) than in the present study (5 of 14 patients).

Discussion

A platelet aggregability measurement system using a laser-light scattering method that can detect increases in the size of small-sized platelet aggregates, which can not be evaluated with conventional methods, has been developed.^{17,18} The new device has proved useful clinically through its ability to detect the enhanced responsiveness of platelets to low concentrations of physiological agents in various diseases.^{17–19} The 2 phases of platelet aggregate formation (ie, the number of large-sized aggregates increases with a concomitant decrease in the number of small-sized platelet aggregates) represent the 'primary' and 'secondary' aggregation processes and so the production of small-sized platelet aggregates is very important for thrombus formation. With a high concentration, the number of large-sized platelet aggregates is too high to enable evaluation of different sized-platelet aggregates. In the present study we used a low concentration of ADP to investigate the most sensitive platelet aggregates, which may be why there were not many large-sized platelet aggregates.

Using the laser-light scattering method, we examined the platelet aggregability in patients with acute coronary syndromes, and the relationship between platelet aggregability on admission and after treatment. The number of small-sized platelet aggregates after treatment decreased significantly in patients with acute coronary syndromes, but was still significantly higher than the number in the stable exertional angina group and the control group. Coronary angiography shows that thrombus still exists in the infarct-related vessel within 1 month of acute myocardial infarction,²³ reflecting the persistence of a state of chronic hyperaggregability in patients with acute coronary syndromes after clinical stabilization. Using this laser-light scattering method, we recently reported that small-sized platelet aggregates increased after anginal attacks in patients with coronary spastic angina pectoris.²⁴

Miecke et al reported that spontaneous platelet aggregation in vitro was a useful biological marker for the prediction of coronary events and mortality in survivors with recent myocardial infarction.²⁵ Recently, Goto et al reported that shear-induced platelet aggregation was enhanced in patients with acute myocardial infarction,²⁶ and previous studies reported that platelet aggregability increased in patients with diabetes mellitus.^{27,28} This is one of the reasons for the slightly higher number of small-sized platelet aggregates in the patients with stable exertional angina in our preliminary study that examined the influence of aspirin. In the present study, there were no significant differences in the number of the patients with diabetes mellitus between the acute coronary syndromes group and the stable exertional angina group. We consider that the difference in the number of

small-sized platelet aggregates reflected the pathological difference between these 2 groups.

In the present study, 81 mg/day aspirin suppressed production of large-sized platelet aggregates, but did not influence the production of small- or medium-sized aggregates. Tohgi et al reported that, using ADP 1.0 μ mol/L as an aggregating reagent, 330 mg/day of aspirin suppressed production of large-sized platelet aggregates, but not that of small- or medium-sized platelet aggregates.²⁹ In general, aspirin can reduce cardiac mortality in patients with acute coronary syndromes²² and its mechanism may be not so much reducing the number of small-sized platelet aggregates, but rather reducing the process by which large-sized platelet aggregates form from small-sized ones. Furthermore, in platelet aggregation using ADP, thromboxane A₂ does not influence small-sized platelet aggregates.¹⁸ Roth et al demonstrated that exposure of platelets to aspirin inhibits cyclooxygenase and thus decreases production of thromboxane A₂.³⁰ Previous studies showed that ticlopidine's antiplatelet action is relatively specific against ADP aggregation,³¹ and Tohgi et al also reported that the number of small-sized platelet aggregates after 0.5 μ mol/L ADP stimulation, and of medium and large-sized platelet aggregates after 1.0 μ mol/L ADP stimulation, was found to be lower in patients treated with 200 mg/day of ticlopidine than in untreated patients.²⁹ Therefore, we considered that ticlopidine influenced the production of small-sized platelet aggregates, and excluded the use of antiplatelet drugs except for aspirin. As it is thought that the decrease in the number of small-sized platelet aggregates after treatment reflects stabilization of condition of disease in patients with acute coronary syndromes, the present findings indicate that the formation of small-sized platelet aggregates may be a sensitive marker for platelet hyperaggregability.

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