Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

Characterization of the Antiplatelet Effect of Aspirin at Enrollment and After 2-Year Follow-up in a Real Clinical Setting in Japan

Tomoyuki Ikeda, MD; Ryoji Taniguchi, MD; Shin Watanabe, MD; Mitsunori Kawato, MD; Hirokazu Kondo, MD; Ryutaro Shirakawa, PhD; Keiichiro Yamane, MD; Masanao Toma, MD; Toshihiro Tamura, MD; Kanako Takahashi; Haruyo Watanabe; Yuka Yoshikawa; Arata Tabuchi, MD*; Toru Kita, MD; Takeshi Kimura, MD; Hisanori Horiuchi, MD

Background: Aspirin is an antiplatelet drug widely used for the prevention of cardiovascular diseases. It has been reported that some patients who exhibit a reduced antiplatelet effect of aspirin have higher cardiovascular risk. It is still controversial whether the antiplatelet effect of aspirin diminishes after a few years of treatment. This study aimed to evaluate the antiplatelet effect of aspirin and its 2-year change in Japanese patients.

Methods and Results: Collagen-induced platelet-aggregability was measured at enrollment by conventional optical aggregometer in 239 patients undergoing antiplatelet therapy with aspirin alone. Among them, 167 patients were evaluated after 2 years. Whole blood aggregability based on the screen-filtration method was also evaluated. Optical aggregometer studies showed that 27% of patients were low-responders. Multivariate analyses revealed that female sex and non-use of calcium-channel blockers were associated with low responsiveness. The antiplatelet effect of aspirin did not decrease after 2 years. Similar data were obtained with the whole blood aggregometer.

Conclusions: In this Japanese patient group, 27% were low-responders to aspirin, and the antiplatelet effect of aspirin did not decrease after a 2-year interval. (*Circ J* 2010; **74:** 1227–1235)

Key Words: Antiplatelet therapy; Aspirin; Cardiovascular risk

Aspirin is an antiplatelet agent that is widely used for the prevention of cardiovascular diseases.[1] Aspirin inhibits platelet cyclooxygenase-1 and it prevents the formation of the pro-aggregatory substance, thromboxane A2. It has been reported that some patients who exhibit a reduced antiplatelet effect of aspirin have higher cardiovascular risk.[2–4] Another issue that has been raised regarding aspirin therapy concerns the long-term change in its antiplatelet effect. One study has shown that the antiplatelet effect of aspirin decreased after 2 years,[5] although conflicting results have also been reported.[6] Therefore, this issue needs to be solved at the earliest opportunity.

Editorial p1077

Platelet aggregability is often evaluated using the optical aggregometer developed by Born et al.[7] This method contains multiple steps, including preparation of platelet-rich plasma (PRP), and is difficult to perform in the real clinical setting because of its complexity. To overcome this, several methods of measuring platelet aggregability directly in whole blood have been developed. One of them is the screen filtration pressure (SFP) method,[8–10] in which whole-blood aggregability is analyzed by measuring the absorbing pressure of agonist-stimulated whole blood through a microsieve. We have shown in previous study of healthy subjects that the antiplatelet effect of aspirin can be clearly evaluated with this method.[11]

Several million Japanese patients are currently under aspirin therapy, mainly for the secondary prevention of ischemic heart disease or prevention of stent thrombosis.[12] However, only a few studies have evaluated the antiplatelet effect of aspirin in Japanese patients.[13,14] Moreover, the long-term change in the antiplatelet effect of aspirin has not been evaluated in Japanese patients. In the present study, we evaluated the prevalence of low-responders to aspirin and the factors affecting platelet aggregability under aspirin therapy in Japanese patients. Furthermore, we evaluated change in the antiplatelet effect of aspirin after a 2-year interval in a real clinical setting in Japan.

Received November 30, 2009; accepted January 28, 2010; released online April 10, 2010 Time for primary review: 21 days
Departments of Cardiovascular Medicine, *Geriatric Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan
Mailing address: Hisanori Horiuchi, MD, Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: horiuchi@kuhp.kyoto-u.ac.jp
ISSN-1346-9843 doi:10.1253/circj.CJ-09-0927

Methods

Study Population and Procedures

This study was approved by the Ethics Committee, Faculty of Medicine, Kyoto University. Between May 2005 and May 2007, patients with high cardiovascular risk under antiplatelet therapy with aspirin, ticlopidine, clopidogrel, cilostazol, sarpogrelate, beraprost sodium, dipyridamole, or ethyl icosapentate for more than 60 days were enrolled in the Anti-Platelet Therapy Effectiveness Study (APTEST) database. Inclusion criteria were: under antiplatelet therapy >6 months and having at least one of the following criteria: age >70 years at enrollment; diabetes mellitus; prior myocardial infarction or angina before at least 6 months; prior percutaneous coronary intervention or coronary artery bypass graft before at least 6 months; prior cerebral infarction before at least 6 months; abdominal aorta aneurysm (≥4 cm in diameter); arteriosclerosis obliterans (Fontain grade ≥2); common or internal carotid artery stenosis (≥70%); obstructive sleep apnea syndrome (apnea–hypopnea index ≥20); body mass index ≥30 kg/m^2; any prior thromboembolism before at least 6 months. Exclusion criteria of the APTEST were having one or more of the following: prior myocardial infarction, angina or cerebral infarction within the last 6 months; age >81 years at enrollment; serum creatinine level ≥2.0 mg/dl; known liver cirrhosis; treatment with warfarin or corticosteroids; hemoglobin level ≤10 g/dl; platelets ≤15,000/mm^3 or ≥35,000/mm^3; active internal bleeding. Blood sampling was performed at enrollment and after 2 years (range, 595–948 days, mean 752±59 days). Among 440 patients enrolled in the APTEST database, data from 239 patients treated with aspirin alone as an antiplatelet drug were analyzed for this study. Patients who met the inclusion criteria but took no antiplatelet drugs (n=25) were evaluated as controls after being recruited separately from APTEST during the same period.

Patients were considered as hypertensive if their blood pressure >140/90 mmHg on at least 2 measurements or if they were being treated with antihypertensive drugs. Patients were considered as hypercholesterolemic if the serum low-density lipoprotein cholesterol level calculated by the Friedewald's equation, serum high-density lipoprotein level, or serum triglycerides level was >140 mg/dl, <40 mg/dl, or >150 mg/dl, respectively or if they were being treated with lipid-lowering agents. Patients were considered as having diabetes mellitus if they were receiving treatment with antidiabetic drugs or insulin. Patients on dietary treatment alone who met the diagnostic criteria listed in the "Report of the Committee of Japan Diabetes Society (JDS) on the classification and diagnostic criteria of diabetes mellitus"[15] were also considered to have diabetes.

Measurement of PRP Aggregation

Fasting blood samples were collected using a 21G needle, with tourniquet, into a polyester tube containing a final solution of 0.313% sodium citrate. PRP was prepared by centrifugation at 200 g at 25°C for 15 min and platelet-poor plasma was prepared by centrifugation at 2,000 g at 25°C for 10 min. Because the aggregability of both the PRP and whole blood was stable during the 60–120 min after blood collection, we analyzed aggregability during this period.[11] Platelet aggregation of PRP induced by 0.1, 0.4, 1.6, 6.4 and 25.6 mg/L horse tendon collagen (Holm, Germany) was evaluated by optical aggregometer, MCM HEMA TRACER 212® (MC medical, Tokyo, Japan). The maximal platelet aggregation rates (MARs) and the platelet-aggregation threshold index (PATI), defined as the putative agonist-concentration giving 50% aggregation based on the light transmission rate at 5 min after stimulation, were evaluated as described previously.[11] A lower PATI indicates higher aggregability. PATI evaluating PRP aggregability is described here as the PRP-PATI.

Measurement of Whole Blood Aggregation

Whole-blood aggregability was measured with a whole-blood aggregometer using the SFP method (WBA-Neo®: ISK, Tokyo, Japan).[8–10] The reaction was started by the addition of 20 μl of agonist solution to 180 μl of whole blood while constantly stirring at 37°C. The final concentrations of collagen were 0.1, 0.4, 1.6, and 6.4 mg/L. At 5 min after stimulation, the absorbing pressure of aggregated whole blood was measured through a microsieve with 30×30 μm windows, and a negative pressure of –130 mmHg was defined as 100% of aggregation and –6 mmHg as 0%. The latter deviation from 0 mmHg was designated because of the viscosity of unstimulated whole blood. The PATI was calculated and defined as the WB-PATI.[11] A lower WB-PATI also meant higher aggregability.

Laboratory Testing

General serum values were measured by the SRL Laboratory Co (Tokyo, Japan). Serum adiponectin concentrations were measured using a commercially available enzyme-linked immunosorbent assay kit (Otsuka Life Science Initiative, Tokyo, Japan) according to the manufacturer's instruction.

Statistical Analysis

All statistical analyses were performed using SAS Institute Inc JMP version 7 (Cary, NC, USA). The PRP- and WB-PATI values are expressed as "medians (interquartile ranges)" because these values were not normally distributed. All the other continuous variables were expressed as the "mean±SD". Intergroup comparison of continuous variables was analyzed by Student's t-test or Wilcoxon 2-sample test if not normally distributed. Categorical variables were presented as frequency counts and percentages, and intergroup comparisons of categorical variables were analyzed by chi-square test. The comparisons of the values at enrollment and after 2 years were performed by paired t-test or Wilcoxon signed-rank test if not normally distributed. A P-value less than 0.05 was considered to be statistically significant. Pearson's correlation coefficient was used to assess the correlation between the MARs at enrollment and after 2 years, between the log-transformed values of PRP-PATI and WB-PATI, between the PRP-PATI values at enrollment and after 2 years, and between the WB-PATI values at enrollment and after 2 years. Because the PRP-PATI and WB-PATI values were not normally distributed, they were log-transformed in order to use Pearson's correlation coefficient. A multivariate logic regression analysis was used to identify clinical predictors of aspirin low-responders.

Results

Clinical Characteristics of the Study Patients

Among the 440 patients enrolled in the APTEST database, results from 239 patients under treatment with aspirin alone as an antithrombotic drug were analyzed. These 239 patients whose platelet aggregability was evaluated at enrollment were defined as the aspirin group 1.

Among the evaluated 239 patients under aspirin therapy at enrollment, we had data for 180 patients after the 2-year

Table 1. Clinical Characteristics and Medications of the Study Patients

	Aspirin group 1 (n=239)	Aspirin group 2 (n=167)	Control group (n=25)
General characteristics			
Age, years	68.5±8.0	68.1±8.1	68.1±9.0
Body mass index (kg/m^2)	23.4±3.1	23.7±3.1†	22.2±3.0
Male	166 (69%)††	119 (71%)††	6 (25%)
Risk factors			
Hypertension	202 (85%)	142 (85%)	18 (75%)
Diabetes	106 (44%)††	71 (43%)††	3 (13%)
Hyperlipidemia	184 (77%)	136 (81%)	17 (71%)
Smoking habit	31 (13%)	23 (14%)†	1 (4%)
Family history of cardiovascular disease	86 (37%)	58 (36%)	7 (33%)
No. of risk factors			
0	4 (2%)	3 (2%)	0 (0%)
1	25 (11%)	17 (11%)	5 (26%)
2	79 (34%)	55 (34%)	8 (42%)
3	84 (37%)	57 (35%)	6 (31%)
4	33 (14%)	25 (16%)	0 (0%)
5	5 (2%)	4 (2%)	0 (0%)
Ischemic heart disease	151 (63%)††	108 (65%)††	2 (8%)
Medications			
β-blocker	77 (32%)	55 (33%)	5 (21%)
Calcium-channel blocker	142 (59%)	106 (63%)	14 (58%)
ACE inhibitor	31 (13%)	26 (17%)	1 (4%)
ARB	98 (41%)	69 (41%)	6 (25%)
Statin	162 (68%)	122 (73%)	15 (63%)
Aspirin dosage (mg/day)	96±28	95±27	

Continuous variables are expressed as mean±SD. Categorical variables are presented as frequency counts (percentages).
†P<0.05 vs Control group, ††P<0.01 vs Control group.
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

Table 2. Hematologic Data of Study Patients

	Aspirin group 1	Aspirin group 2	Control group
Platelet number (×10^3/μl)	215±43	218±45	214±44
Red blood cell count (×10^4/μl)	449±44	452±42	442±39
Creatinine (mg/dl)	0.83±0.21††	0.83±0.22††	0.68±0.20
Total cholesterol (mg/dl)	181±29	179±29†	192±29
Triglycerides (mg/dl)	113±64	116±68	102±49
HDL-cholesterol (mg/dl)	52±12	52±12	57±12
Fasting blood glucose (mg/dl)	113±29	111±27	106±23
HbA_{1c} (%)	6.26±1.13	6.17±1.09	5.97±0.77
Uric acid (mg/dl)	5.7±1.2	5.8±1.3	5.3±1.3
BNP (pg/ml)	79.0±117.8	66.1±85.7	54.0±40.0
Adiponectin (μg/ml)	9.7±7.8	9.7±7.3	10.2±7.6
High sensitive CRP (ng/dl)	1,813±9,524	2,085±11,332	1,363±3,191

†P<0.05 vs Control group, ††P<0.01 vs Control group.
HDL, high-density lipoprotein; HbA_{1c}, hemoglobin A_{1c}; BNP, brain natriuretic peptide; CRP, C-reactive protein.

interval because 24 patients were lost to follow-up during this period, 4 patients started treatment with warfarin, an exclusion criterion of the study during follow-up, 11 patients did not agree to blood sampling, 4 patients died and 16 patients were not evaluated for technical reasons. We also excluded 5 patients who started taking other antiplatelet drugs during the 2-year period and 8 patients who had cardiovascular events. After these exclusions, we analyzed the data for remaining 167 patients to evaluate the change in the antiplatelet effect of aspirin at 2 years (595–948 days, 752±59 (mean±SD) days). The 167 patients whose platelet aggregability was evaluated at both enrollment and after 2 years were defined as the aspirin group 2. The platelet aggregability of the 25 control patients was also analyzed at enrollment and after 2 years.

The baseline characteristics and medications of each group are shown in Table 1. The baseline characteristics of the aspirin groups 1 and 2 did not notably differ from each other.

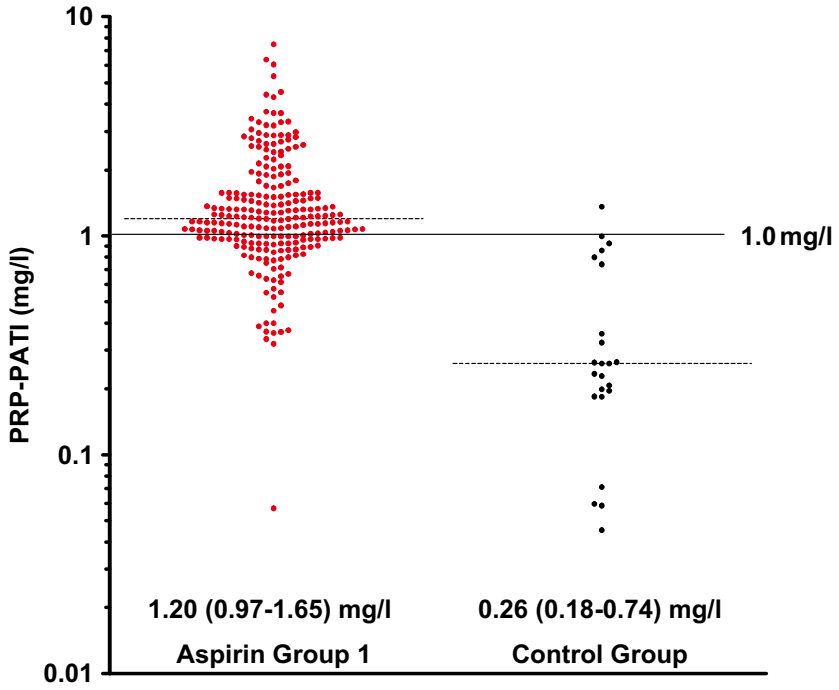

Figure 1. PRP-PATI values of the aspirin group 1 (**Left**) and the control group (**Right**) at enrollment. The 239 patients under aspirin therapy whose platelet aggregability was evaluated at enrollment were defined as the aspirin group 1. The dotted line represents the median value. The PRP-PATI values of each group are expressed as "median (interquartile range)". PRP-PATI 1.0 mg/L was determined as the cut-off value for distinguishing aspirin intake in our previous study,[11] so in the present study patients with PRP-PATI <1.0 mg/L were defined as low-responders to aspirin. The PRP-PATI values are plotted on a logarithmic scale. PRP-PATI, platelet-rich plasma–platelet-aggregation threshold index.

Table 3. Analysis for Aspirin Low-Responders Defined by PRP-PATI

	Low responders (n=67)	Others (n=172)	Univariate P value	Multivariate P value	OR (95%CI)
General characteristics					
Age (years)	69.2±1.0	68.5±0.6	0.54		
Height (m)	1.60±0.01	1.62±0.01	0.07		
Body weight (kg)	62.0±0.9	60.1±1.4	0.25		
Body mass index (kg/m^2)	23.4±0.4	23.4±0.2	0.93		
Male	53%	75%	0.0018	0.001	2.88 (1.53–5.48)
Risk factors					
Hypertension	81%	86%	0.32		
Diabetes	42%	46%	0.56	0.86	1.06 (0.57–1.96)
Hyperlipidemia	69%	80%	0.087	0.82	1.05 (0.46–2.61)
Family history	38%	36%	0.88		
Smoking habit	10%	14%	0.67	0.92	1.05 (0.42–2.87)
Ischemic heart disease	55%	66%	0.14		
Medications					
β-blocker	32%	31%	0.7		
Calcium-channel blocker	45%	65%	0.005	0.01	2.17 (1.19–4.01)
ACE inhibitor	10%	14%	1		
ARB	39%	42%	0.66		
Statin	57%	72%	0.03	0.11	1.91 (0.86–4.23)
Aspirin dosage (mg/day)	92±3	98±2	0.12		

PRP-PATI, platelet-rich plasma–platelet-aggregation threshold index; OR, odds ratio; CI, confidence interval. For other abbreviations see Table 1.

The control group contained more females than the aspirin groups and the patients in the control group had fewer coronary risk factors than those in the aspirin groups. There were no significant differences in medications, except for aspirin use, between the aspirin groups and the control group. Standard laboratory data of the 2 aspirin groups and the control group were similar, except for slight but statistically significant changes in the levels of serum creatinine (aspirin groups 1 and 2 vs control group) and total cholesterol (aspirin group 2 vs control group) (**Table 2**).

Prevalence of Aspirin Low-Responders in Japanese Patients

We have previously reported that in a study of healthy subjects the PRP-PATI values were well correlated with the

Table 4. Comparisons of Data Obtained at Enrollment and After 2 Years

	At enrollment	After 2 years	P value
Platelet number (×10³/μl)	218±45	214±44	0.1
Red blood cell count (×10⁴/μl)	452±42	452±48	0.98
Creatinine (mg/dl)	0.83±0.22	0.88±0.27	<0.0001
Total cholesterol (mg/dl)	179±29	178±30	0.51
Triglycerides (mg/dl)	116±68	115±67	0.71
HDL-cholesterol (mg/dl)	52±12	52±13	0.82
Fasting blood glucose (mg/dl)	111±27	111±34	0.63
HbA_{1c} (%)	6.17±1.09	6.08±0.98	0.03
Uric acid (mg/dl)	5.8±1.3	5.9±1.2	0.25
BNP (pg/ml)	66.1±85.7	65.4±78.6	0.9
High-sensitivity CRP (ng/ml)	2,085±11,332	1,542±6,961	0.6

For abbreviations see Table 2.

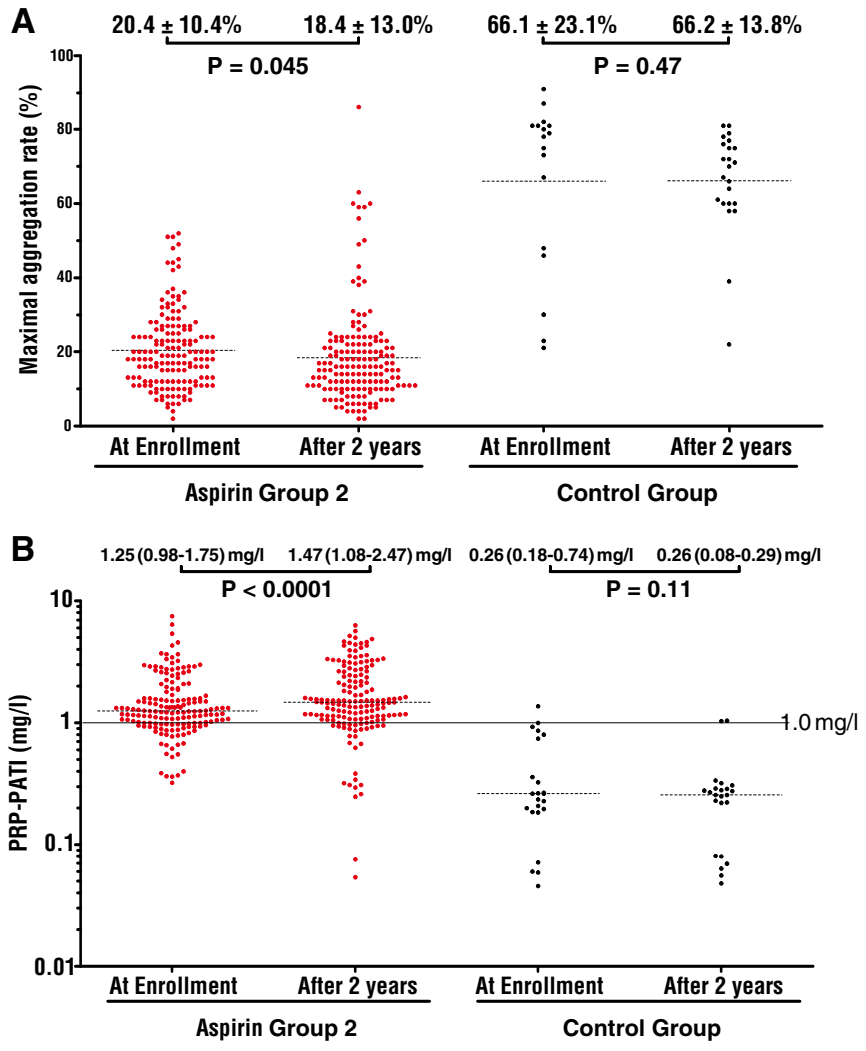

Figure 2. Platelet aggregability at enrollment and after 2 years in 167 patients under aspirin therapy defined as aspirin group 2. (**A**) MARs induced by 0.4 mg/L collagen at enrollment and after 2 years were compared. The dotted line of each group represents the mean values. The MARs are expressed as the means ± SD. (**B**) PRP-PATI values at enrollment and after 2 years were compared. The dotted line of each group represents the median values. PRP-PATI values are expressed as "median (interquartile range)" and plotted on a logarithmic scale. The patients with PRP-PATI <1.0 mg/L were defined as low-responders to aspirin in this study. PRP-PATI, platelet-rich plasma–platelet-aggregation threshold index; MARs, maximal aggregation rates.

MARs, and that the PRP-PATI could be a better indicator for distinguishing aspirin intake than the MARs.[11] In that report, we proposed that the cut-off value for distinguishing aspirin intake was the PRP-PATI value of collagen concentration at 1.0 mg/L (specificity 91%, sensitivity 100%). Because the platelet aggregability of the patients with a PRP-PATI <1.0 mg/L was within the same level as that of patients not taking aspirin, we defined patients with PRP-PATI <1.0 mg/L as low-responders to aspirin in this study.

The PRP-PATI value of the 239 patients in the aspirin group 1 was 1.20 (0.97–1.65) (median (interquartile range)) mg/L while that of the control group was 0.26 (0.18–0.74) mg/L.

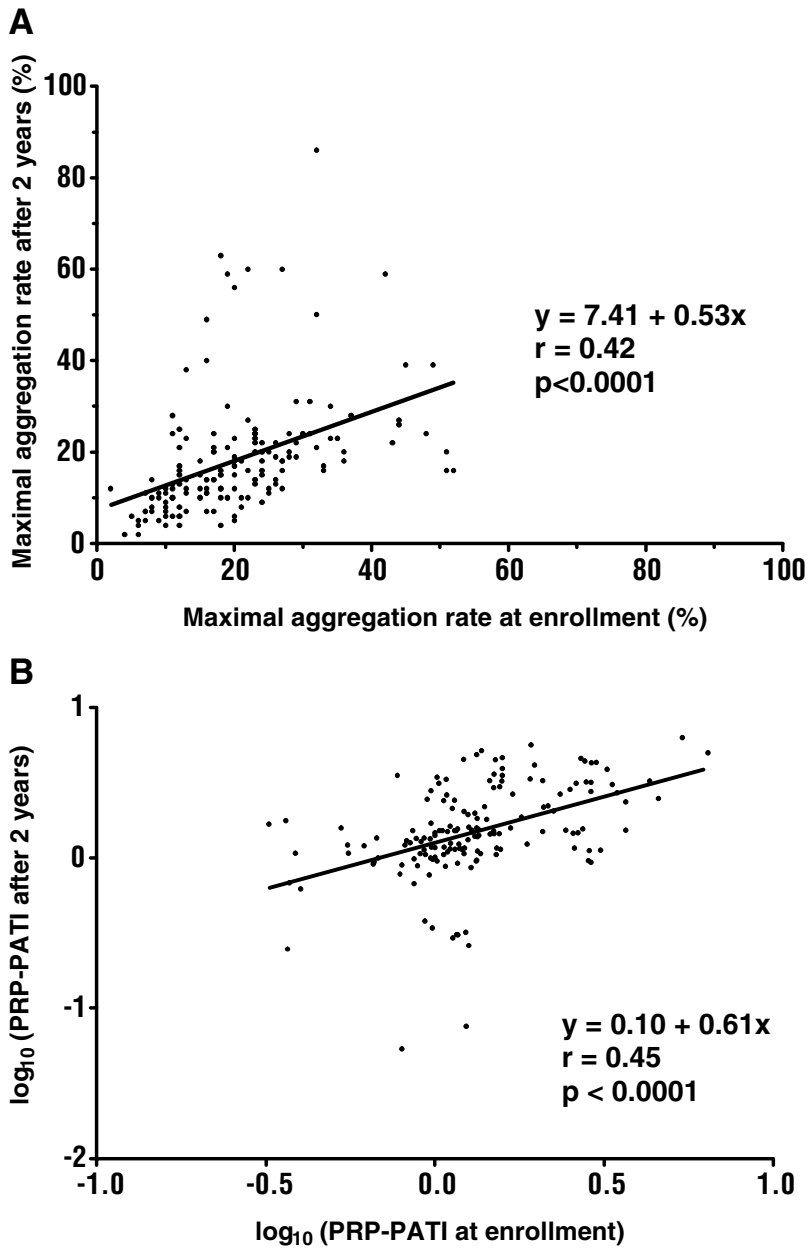

Figure 3. Correlation between platelet aggregability at enrollment and after 2 years. (**A**) Scatter plots of the association between the MARs at enrollment and after 2 years. (**B**) Scatter plots of the association between the PRP-PATI values at enrollment and after 2 years. The PRP-PATI values were log-transformed. PRP-PATI, platelet-rich plasma–platelet-aggregation threshold index; MARs, maximal aggregation rates.

Among the 239 patients under aspirin therapy, 67 (27%) had a PRP-PATI value for collagen <1.0 mg/L (ie, low-responders to aspirin) and in the control group, 24 (96%) had PRP-PATI values <1.0 mg/L (**Figure 1**).

Characteristics of Patients With Insufficient Antiplatelet Effect of Aspirin

The 67 patients who had PRP-PATI values <1.0 mg/L were defined as low responders. Univariate analysis revealed that female sex, non-use of calcium-channel blockers (CCB), and non-use of statins were associated with low response to aspirin (**Table 3**). Because hyperlipidemia,[16] diabetes mellitus[17] and smoking habit[18] are reportedly associated with low response to aspirin, multivariable analysis was performed using the variables that were significantly different by the univariate analysis as well as these 3 variables. Female sex (odds ratio (OR) 2.88, 95% confidence interval (CI) 1.53–5.48, P=0.001) and non-use of CCBs (OR 2.17, 95%CI 1.19–4.01, P=0.01) were significantly associated with a low response to aspirin (**Table 3**).

Evaluation of the Antiplatelet Effect of Aspirin at Enrollment and After 2 Years

We evaluated the change in the antiplatelet effect of aspirin after a 2-year interval in the 167 patients in the aspirin group 2. The general laboratory data at enrollment and after 2 years were similar, except for serum creatinine levels and hemoglobin A_{1c} levels, although the changes were minimal (**Table 4**).

In aspirin group 2, the MARs induced by 0.4 mg/L collagen was 20.4±10.4% at enrollment and 18.4±13.0% after 2 years and the respective values for the control group were 66.1±23.1% and 66.2±13.8% (**Figure 2A**). Thus, platelet aggre-

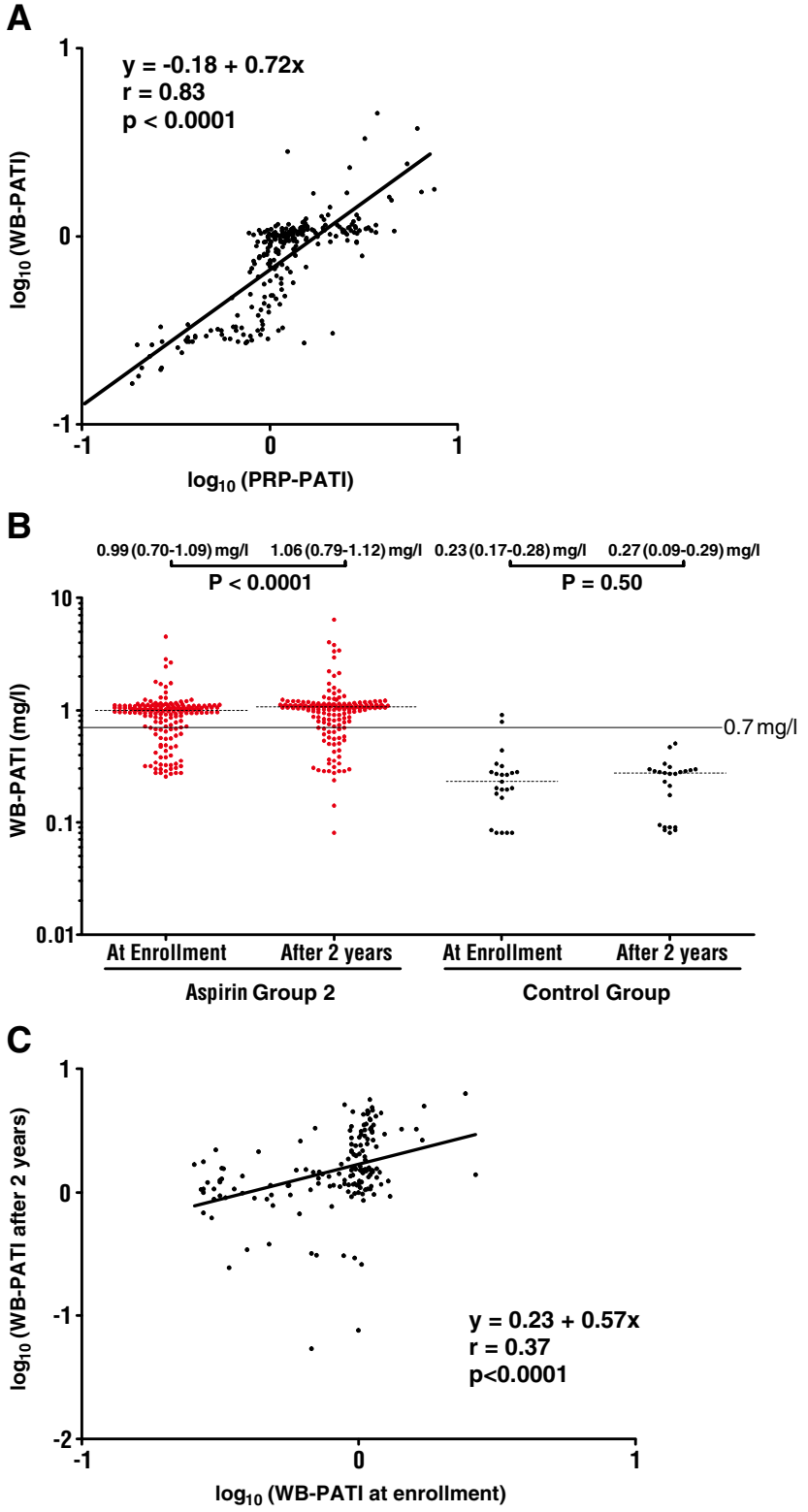

Figure 4. Analysis using the WB-PATI values measured with the whole-blood aggregometer. (**A**) Scatter plots of the association between the PRP-PATI and WB-PATI values at enrollment. Both values were log-transformed. (**B**) WB-PATI values at enrollment and after 2 years. The 167 patients under aspirin therapy whose platelet aggregability was evaluated both at time points were defined as aspirin group 2 and their WB-PATI values were compared. The dotted line of each group represents the median values. The WB-PATI values were plotted on a logarithmic scale and expressed as "medians (interquartile ranges)". Patients with WB-PATI values <0.7 mg/L were defined as low-responders to aspirin in this study. (**C**) Scatter plots of the association between the WB-PATI values at enrollment and after 2 years. The WB-PATI values were log-transformed. WB-PATI, whole blood–platelet-aggregation threshold index.

gability under aspirin therapy evaluated with MARs decreased, rather than increased, after the 2-year interval.

The PRP-PATI values increased significantly from 1.25 (0.98–1.75) mg/L at enrolment to 1.47 (1.08–2.47) mg/L after the 2-year interval, whereas in the control group they did not change significantly: 0.26 (0.18–0.74) mg/L and 0.26 (0.08–0.29) mg/L, respectively (**Figure 2B**). Thus, in the aspirin group 2, platelet aggregability did not increase but even decreased during the 2-year interval.

To analyze the change in the aspirin effect in individual

patients, the correlation between platelet aggregability at enrollment and after 2 years was assessed by Pearson's correlation coefficient. The MARs at enrollment were positively associated with those after 2 years (r=0.42, P<0.0001; Figure 3A). The log-transformed values of the PRP-PATI values at enrollment were also associated with those after 2 years (r=0.45, P<0.0001; Figure 3B). Thus, the antiplatelet effect of aspirin appears to be individual-specific over 2 years.

Analysis With Whole-Blood Aggregometer

In this study, we also analyzed whole-blood aggregability based on the SFP method, a procedure that does not require PRP preparation. The WB-PATI values correlated well with the PRP-PATI values in our previous study of healthy subjects.[11] In order to clarify whether it is also the case in the clinical setting, we assessed the correlation between the WB-PATI and PRP-PATI values in this study. As shown in Figure 4A, the log-transformed values of the WB-PATI values of the all patients evaluated at enrollment (aspirin group 1+control group) correlated well with the PRP-PATI values (r=0.83, P<0.0001). Thus the WB-PATI values correlated well with the PRP-PATI values not only in healthy subjects but also in patients in a real clinical setting.

The cut-off value of the WB-PATI value to detect aspirin intake has been determined as 0.7 mg/L (specificity 94%, sensitivity 97%) in our previous study of healthy subjects.[11] Among the 239 patients under aspirin therapy, 64 (26%) had a WB-PATI value for collagen <0.7 mg/L, making them potential low responders to aspirin. The prevalence was similar to that defined by the PRP-PATI.

Furthermore, the WB-PATI values for collagen at enrollment and after 2 years were 0.99 (0.70–1.09) mg/L and 1.06 (0.79–1.12) mg/L, respectively, indicating that the antiplatelet effect of aspirin after 2 years (P<0.0001) also increased in the analysis using the whole-blood aggregometer (Figure 4B). The log-transformed values of the WB-PATI values at enrollment were positively associated with those after 2 years (r=0.37, P<0.0001; Figure 4C).

Thus, the results evaluated by the PRP-PATI values were reconfirmed by the analysis of the WB-PATI values regarding the prevalence of low-responders to aspirin and the 2-year change in the aspirin effect.

Discussion

In the present study we demonstrated a prevalence of low-responders to aspirin of 27% in Japanese patients by our criterion, and that female sex and non-use of CCBs are associated with a low response to aspirin. Furthermore, the antiplatelet effect of aspirin did not decrease after a 2-year period. The data obtained by whole-blood aggregometer exhibited similar results.

Although aspirin is an antiplatelet drug widely used for the prevention of cardiovascular diseases, all patients do not exhibit a uniform antiplatelet effect and a low response to aspirin is associated with a higher cardiovascular risk.[2–4,19] Therefore, a current issue is the variability of the antiplatelet effect of aspirin and also which factors influence that effect. We previously reported that 90% of healthy subjects have a PRP-PATI >1.0 mg/L on day 14 after starting aspirin intake.[11] Therefore, in the present study, we defined patients with a PRP-PATI <1.0 mg/L as low-responders to aspirin. Using this cut-off point, the proportion of patients with insufficient antiplatelet effect of aspirin was 27%. In other words, these patients exhibited a platelet aggregability that was almost the same as that of patients not taking aspirin. Thus, approximately 25% of Japanese patients in the real clinical setting might be low responders.

Several factors could cause a low response to aspirin. In previous studies, noncompliance, drug interactions, mainly with nonsteroidal antiinflammatory drugs, inadequate dosing, female sex, smoking, hyperlipidemia, and diabetes mellitus have been reported as risk factors for a low response to aspirin.[16–18,20–22] In the present study, multivariate analysis of the PRP-PATI values revealed that female sex (OR 2.88, 95%CI 1.53–5.48, P=0.001) and non-use of CCBs (OR 2.17, 95%CI 1.19–4.01, P=0.01) were significantly associated with a decreased antiplatelet effect of aspirin (Table 3). CCBs are reported to inhibit platelet aggregation through enhancing nitric oxide synthase activity,[23,24] so they might enhance the antiplatelet effect of aspirin through that mechanism. Further study of the influence of CCBs on the antiplatelet effect of aspirin is required. In the present study, there were not significant differences between the dosage of aspirin, smoking habit, hyperlipidemia, and diabetes mellitus.

There are a few previous studies that have analyzed the long-term effect of aspirin. Berglund and Wallentin[6] measured the platelet aggregability of 193 patients with unstable angina before and after 5 days, and 1, 12, 18, and 24 months of aspirin therapy. Collagen-induced platelet aggregability was similarly inhibited throughout the study period without attenuation. On the other hand, Pulcinelli et al[5] analyzed the long-term effect of aspirin in 150 patients with recent atherothrombosis before and after 2, 6, 12, and 24 months of aspirin therapy The maximal aggregation rates were 88.2±21.8% before treatment, 37.9±24.4% after 2 months, 46.1±27.1% after 6 months, 48.7±27.6% after 12 months, and 61.9±23.9% after 24 months. In their study, the collagen-induced platelet aggregation after 2 years was significantly higher than that observed at 2 months after starting aspirin. Therefore, physicians have become suspicious about the long-term effect of aspirin, which is quite an important clinical issue, because we need to improve or change aspirin therapy if it antiplatelet effect gradually decreases with time. In the present study, however, we demonstrated that the antiplatelet effect of aspirin did not deteriorate, and even improved, after a 2-year follow-up, which indicates that when we use aspirin for long-term prevention of cardiovascular disease, we might not have to consider changing aspirin for other antiplatelet drugs to avoid the deterioration in antiplatelet effect. In this study we evaluated patients who took aspirin for more than 6 months, whereas the aforementioned 2 studies addressed the first 24 months of aspirin therapy, so it may be difficult to compare these studies directly. Nevertheless, in terms of the long-term change in aspirin effect, our study clearly demonstrated that there was no decrease in the effect over 2 years. The reason why the antiplatelet effect of aspirin increased is unclear, but it may be related to lifestyle change and/or improved drug compliance during the 2-year follow-up.

In the present study, we also analyzed whole-blood aggregability based on the SFP method, which has fewer preparation steps than the PRP preparation and so could be easier and more feasible to use in the clinical setting. The WB-PATI values measured by the whole-blood aggregometer correlated well with the PRP-PATI values by conventional optical aggregometer in our previous study of healthy subjects.[11] In the present study, we evaluated the effect of aspirin with this method in a real clinical setting and the WB-PATI values were also well correlated with the PRP-PATI values (Figure 4A). The ratio of patients with an insufficient antiplatelet effect

of aspirin was 26% by this method, which was similar to the result using the PRP-PATI values (27%). The WB-PATI at enrollment was higher than that after 2 years, as with the PRP-PATI. Thus, similar results were obtained from analysis of the PRP-PATI values. Evaluation by whole-blood aggregometer could be a more feasible and useful method of monitoring the aspirin effect in actual clinical settings.

Because aspirin is an inhibitor of cyclooxygenase-1, the antiplatelet effect of aspirin might not be directly evaluated by measuring collagen-induced platelet aggregation. However, evaluation of ex-vivo collagen induced platelet aggregation can clearly distinguish pre and post aspirin intake and has been used to evaluate the aspirin effect in many other studies,[16,17,25–27] as well as by us.[11] Therefore, we used collagen as an agonist in this study.

In conclusion, the antiplatelet effect of aspirin could be insufficient in one-fourth of Japanese patients in the real clinical setting. The antiplatelet effect of aspirin might last for at least 2 years in patients who take aspirin for more than 6 months. Furthermore, the WB-PATI was well correlated with the PRP-PATI in the real clinical setting of the present study. Therefore, evaluation of aspirin therapy using the whole-blood aggregometer may feasible in the clinical setting.

Study Limitations

Because we enrolled the patients undergoing aspirin therapy for more than 6 months, the duration of therapy before enrollment may have varied. Another limitation is that this study was performed at a single center.

Acknowledgment

This work was partially supported by a Grant from the Japan Cardiovascular Research Foundation.

Disclosure

The authors declare no conflicts of interest.

References

1. Horiuchi H. Recent advance in antiplatelet therapy: The mechanisms, evidence and approach to the problems. *Ann Med* 2006; **38:** 162–172.
2. Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin "resistance" and risk of cardiovascular morbidity: Systematic review and meta-analysis. *BMJ* 2008; **336:** 195H–198H.
3. Chen WH, Lee PY, Ng W, Tse HF, Lau CP. Aspirin resistance is associated with a high incidence of myonecrosis after non-urgent percutaneous coronary intervention despite clopidogrel pretreatment. *J Am Coll Cardiol* 2004; **43:** 1122–1126.
4. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation* 2002; **105:** 1650–1655.
5. Pulcinelli FM, Pignatelli P, Celestini A, Riondino S, Gazzaniga PP, Violi F. Inhibition of platelet aggregation by aspirin progressively decreases in long-term treated patients. *J Am Coll Cardiol* 2004; **43:** 979–984.
6. Berglund U, Wallentin L. Persistent inhibition of platelet-function during long-term treatment with 75 mg acetylsalicylic-acid daily in men with unstable coronary-artery disease. *Eur Heart J* 1991; **12:** 428–433.
7. Born GVR, Haslam RJ, Goldman M, Lowe RD. Comparative effectiveness of adenosine analogues as inhibitors of blood-platelet aggregation and as vasodilators in man. *Nature* 1965; **205:** 678–680.
8. Ozeki Y, Sudo T, Toga K, Nagamura Y, Ito H, Ogawa T, et al. Characterization of whole blood aggregation with a new type of aggregometer by a screen filtration pressure method. *Thromb Res* 2001; **101:** 65–72.
9. Shio H, Ramwell PW. Prostaglandin-e1 in platelet harvesting: In-vitro study. *Science* 1972; **175:** 536–538.
10. Swank RL, Davis E. Blood cell aggregation and screen filtration pressure. *Circulation* 1966; **33:** 617–624.
11. Tabuchi A, Taniguchi R, Takahashi K, Kondo H, Kawato M, Morimoto T, et al. Action of aspirin on whole blood-aggregation evaluated by the screen filtration pressure method. *Circ J* 2008; **72:** 420–426.
12. Ikari Y, Kotani J, Kozuma K, Kyo E, Nakamura M, Yokoi H, et al on behalf of the J-PMS study group. Assessment of sirolimus-eluting coronary stent implantation with aspirin plus low dose ticlopidine administration: One year results from CYPHER stent Japan Post-Marketing Surveillance registry (J-PMS). *Circ J* 2009; **73:** 1038–1044.
13. Ohmori T, Yatomi Y, Nonaka T, Kobayashi Y, Madoiwa S, Mimuro J, et al. Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity: Involvement of other signaling pathway(s) in cardiovascular events of aspirin-treated patients. *J Thromb Haemost* 2006; **4:** 1271–1278.
14. Fujiwara T, Ikeda M, Esumi K, Fujita TD, Kono M, Tokushige H, et al. Exploratory aspirin resistance trial in healthy japanese volunteers (J-ART) using platelet aggregation as a measure of thrombogenicity. *Pharmacogenomics J* 2007; **7:** 395–403.
15. Kuzuya T, Nakagawa S, Satoh J, Kanazawa Y, Iwamoto Y, Kobayashi M, et al. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res Clin Pract* 2002; **55:** 65–85.
16. Friend M, Vucenik I, Miller M. Platelet responsiveness to aspirin in patients with hyperlipidaemia. *BMJ* 2003; **326:** 82–83.
17. Watala C, Golanski J, Pluta J, Boncier M, Rozalski M, Luzak B, et al. Reduced sensitivity of platelets from type 2 diabetic patients to acetylsalicyclic acid (aspirin): Its relation to metabolic control. *Thromb Res* 2004; **113:** 101–113.
18. Hung J, Lam JYT, Lacoste L, Letchacovski G. Cigarette-smoking acutely increases platelet thrombus formation in patients with coronary-artery disease taking aspirin. *Circulation* 1995; **92:** 2432–2436.
19. Pamukcu B, Oflaz H, Onur L, Midilli K, Yilmaz G, Yilmaz E, et al. Relationship between the serum scd40l level and aspirin-resistant platelet aggregation in patients with stable coronary artery disease. *Circ J* 2008; **72:** 61–66.
20. Gum PA, Kottke-Marchant K, Poggio EC, Gurm H, Welsh PA, Brooks L, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. *Am J Cardiol* 2001; **88:** 230–235.
21. Kurth T, Glynn RJ, Walker AM, Chan KA, Buring JE, Hennekens CH, et al. Inhibition of clinical benefits of aspirin on first myocardial infarction by nonsteroidal antiinflammatory drugs. *Circulation* 2003; **108:** 1191–1195.
22. Gonzalez-Conejero R, Rivera J, Corral J, Acuna C, Guerrero JA, Vicente V. Biological assessment of aspirin efficacy on healthy individuals: Heterogeneous response or aspirin failure? *Stroke* 2005; **36:** 276–280.
23. Chou TC, Li CY, Yen MH, Ding YA. Antiplatelet effect of amlodipine: A possible mechanism through a nitric oxide, mediated process. *Biochem Pharmacol* 1999; **58:** 1657–1663.
24. Wallen NH, Held C, Rehnqvist N, Hjemdahl P. Platelet aggregability in-vivo is attenuated by verapamil but not by metoprolol in patient with stable angina-pectoris. *Am J Cardiol* 1995; **75:** 1–6.
25. Mueller MR, Salat A, Stangl P, Murabito M, Pulaki S, Boehm D, et al. Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. *Thromb Haemost* 1997; **78:** 1003–1007.
26. Buerke M, Pittroff W, Meyer J, Darius H. Aspirin therapy: Optimized platelet inhibition with different loading and maintenance doses. *Am Heart J* 1995; **130:** 465–472.
27. Nishiyama A, Niikawa O, Mohri H, Tsushima M. Timing of antiplatelet effect after oral aspirin administration in patients with sympathetic excitement. *Circ J* 2003; **67:** 697–700.