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Impact of Platelet Reactivity to Adenosine Diphosphate Before Implantation of Drug-Eluting Stents on Subsequent Adverse Cardiac Events in Patients With Stable Angina

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Background: Diverse pharmacological effects of anti-platelet thienopyridines due to individual differences in metabolism have been reported. However, an association between on-treatment platelet reactivity and adverse ischemic events after drug-eluting stent (DES) implantation in Japanese patients has not been fully elucidated.

Methods and Results: A total of 450 consecutive patients on dual anti-platelet therapy (aspirin and ticlopidine) with stable angina who underwent DES implantation were enrolled. Adenosine diphosphate (ADP)-induced platelet aggregation was measured before DES implantation using the screen filtration pressure method. The ADP concentration necessary for 50% aggregation was designated as the platelet aggregation threshold index (PATI). A composite primary endpoint of cardiac death, myocardial infarction, target lesion revascularization (TLR), and stent thrombosis occurring within 1 year after stenting, was evaluated. A PATI value <4.8 μmol/L was defined as high on-treatment reactivity to ADP. The composite primary endpoint occurred in 55 patients (12.2%) in the 1-year-period after DES implantation, and the prevalence was 19.0% and 5.1% in groups with high and low on-treatment reactivity to ADP, respectively, showing a significantly higher prevalence in the high reactivity group (P<0.001). The main event was TLR (18.1% vs. 5.1%, P<0.001).

Conclusions: These data suggested that high on-treatment platelet reactivity to ADP and subsequent occurrence of adverse ischemic events (particularly TLR) were correlated in patients with stable angina who underwent DES implantation. (*Circ J* 2012; **76:** 641–649)

Key Words: Anti-platelet therapy; Drug-eluting stent; Percutaneous coronary intervention; Platelet reactivity to ADP; Revascularization

Aspirin/thienopyridine combination therapy after coronary arterial stenting is an established treatment to prevent stent thrombosis and subsequent ischemic events. However, stent thromboses after coronary stent implantations have repeatedly occurred despite dual anti-platelet therapy (DAPT) being administered.

Recently, an association between low responsiveness to clopidogrel and outcomes (in particular, the development of stent thrombosis) after implantation of drug-eluting stents (DES) has been reported.[1–4] Thienopyridine anti-platelet agents are prodrugs converted to active forms by metabolic means by cytochrome P450 (CYP) in the liver. Hence, their pharmacological effects vary due to individual differences in metabolism and drug interaction. Indeed, heterogeneous pharmacological effects of clopidogrel given via the oral route have been reported.[5,6] It has also been reported that genetic polymorphisms of CYP 2C19 and poor metabolization of clopidogrel are associated, and that subsequent reduction of the inhibitory effects of platelet aggregation leads to stent thrombosis.[7] In a study involving patients who underwent percutaneous coronary intervention (PCI), clopidogrel non-responders accounted for 5–44% of subjects (even though the method of the platelet function test and definition of resistance were different).[8] However, in Japanese patients, fewer studies on the reactivity of platelet aggregation and ischemic

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events after PCI have been reported.[9] Also, there has been no report on ischemic and bleeding events or the reactivity of platelet aggregation under treatment with ticlopidine after coronary DES implantation in patients with stable angina pectoris.

We investigated the association between adenosine diphosphate (ADP)-induced platelet aggregation under treatment with aspirin and ticlopidine in 450 DES-implanted patients with stable angina pectoris and their outcomes at 1 year.

Methods

Subjects

PCI was carried out in 1,757 patients at Tsuchiya General Hospital (Hiroshima, Japan) between January 2007 and December 2008. Of 1,168 PCI cases excluding 493 cases of emergency PCI (acute myocardial infarction (AMI): n=301; unstable angina: n=192) and 96 cases of recent myocardial infarction (MI; including residual stenotic lesions in other branches after AMI), stenting was applied to 986 cases. A sirolimus-eluting stent (SES) or paclitaxel-eluting stent (PES) was implanted in 574 of the 986 cases. From these patients, those undergoing dialysis treatment, with a drug allergy to aspirin or ticlopidine, under clopidogrel treatment, with a hemorrhagic tendency, with anemia (hemoglobin (Hgb) <8.0 g/dl), with thrombocytopenia (platelet count (Plt) <100,000/mm^3), or those undergoing anticoagulant therapy were excluded. The remaining 450 consecutive patients with stable angina pectoris were registered in this study and underwent DES implantation. All patients underwent DAPT with 100 mg or 200 mg of aspirin and 200 mg of ticlopidine for ≥1 week (including patients in whom DAPT had already been done before coronary angiography and PCI was subsequently undertaken after coronary angiography, and those in whom DAPT and subsequent PCI were done after coronary angiography). ADP-induced platelet aggregation was measured before stent implantation. The study protocol and publication were approved by the Ethics Committee of Tsuchiya General Hospital. Written informed consent to undergo stent treatment and blood tests was obtained from all patients.

PCI Procedures

All PCI procedures were undertaken according to standard techniques. A previously described rotational atherectomy was carried out.[10,11] Peri-procedural anti-thrombotic therapy (unfractionated heparin as a bolus of 6,000–10,000 U) was instituted according to the discretion of the Study Director. The PCI procedure was considered to be successful if there was a residual diameter stenosis of <50% in the absence of major peri-procedural complications (death, AMI, emergency bypass surgery).

Analyses of Coronary Lesions

Lesion length, reference diameter, minimal lumen diameter, and percent diameter stenosis were measured using a computerized, automated, edge-detection algorithm (Philips Medical System, Best, The Netherlands).[12] Lesions were classified according to the American Heart Association/American College of Cardiology (AHA/ACC) classification.[13]

Blood Sampling and Measurement of ADP-Induced Platelet Aggregation

Before PCI in a Catheterization Suite, in patients in a fasted resting state, blood was collected through an indwelling sheath in a peripheral artery before the intravenous injection of heparin. Blood in the sheath was discarded, and then 2 ml of peripheral blood was collected. Whole blood was transferred into a blood sampling tube containing 3.8% trisodium citrate and kept at room temperature for 60 min. It was then subjected to the measurement of ADP-induced platelet aggregation using a whole blood analyzer (WBA-Neo Platelet Aggregation Measurement Device; ISK, Tokyo, Japan). This device uses a screen filtration pressure (SFP) method.[14,15] After the addition of a platelet aggregation-inducing substance, the negative pressure produced by aspirating blood through a micromesh filter (diameter, 30 μm; 300 square holes per mm^2) was automatically measured to evaluate platelet aggregation reactivity. The ADP concentration required to cause 50% of maximum secondary aggregation was calculated as the platelet aggregation threshold index (PATI). This method ensures swift completion of measurements, but PATI values alter during the period from blood sampling to measurement.[14] Hence, we took exactly 1 h from blood sampling through measurement initiation by recording the sampling time. ADP could be added at 4 stepwise concentrations simultaneously, but the sample volume from 1 tube was sufficient for only 2 measurements. Moreover, measurement at an ADP concentration >32 μmol/L is difficult with regard to determining the PATI. Thus, the maximum ADP concentration was set at 32 μmol/L.

Clinical Endpoint and Follow up

All patients continued DAPT for 1 year after DES (SES or PES) implantation. Patients were followed up for 1 year after stenting. Clinical events and compliance with anti-platelet drugs were confirmed in medical records and by telephone surveys for all patients. A composite primary endpoint of cardiac death, MI, target lesion revascularization (TLR; including surgical coronary artery bypass and repeat PCI of the original target lesion), and stent thrombosis within 1 year after stenting, was evaluated. MI was defined based on electrocardiographic findings, persistent chest pain in resting states, and elevation of the level of creatinine kinase (CK-MB) isoenzyme to more than 3-fold higher than the normal level. Stent thrombosis was diagnosed based on the definition established by the Academic Research Consortium.[16] TLR was defined as an application of surgical coronary artery bypass or repeat PCI in patients with restenosis of ≥50% upon quantitative coronary angiography (QCA) in whom myocardial ischemia manifested as clinical chest pain or was demonstrated by exercise electrocardiography. A follow-up angiographic study was recommended >9 months after PCI but was done; earlier if clinically indicated. Stent restenosis was defined as stenosis of ≥50% on QCA at follow up. Major hemorrhagic events were defined as hemorrhagic death, intracranial hemorrhage, reduction of the Hgb level by ≥3 g/dl, and conditions requiring a blood transfusion of ≥2 units.

Statistical Analyses

In a large study of the TAXUS-4 trial,[17] the prevalence of major adverse cardiac events (MACE) was 10.8%. In our institute, the prevalence of 1-year MACE after consecutive SES implantation with or without rotational atherectomy was 23.5% and 10.8%, respectively.[10] Therefore, the calculation of the sample size for the present study was based upon the assumption that the prevalence of the composite primary endpoint of cardiac death, MI, TLR, and stent thrombosis was 15% in patients with high on-treatment reactivity to ADP and 8% in patients with low on-treatment reactivity to ADP. A minimal sample size of 440 patients would provide 80% power with a 2-sided alpha value of 0.05. Continuous variables are mean±SD. The Shapiro-Wilk test was used to test for the normal distribution of continuous data. On the basis of the distribution, the Student's t-test or the Wilcoxon rank-sum test was used for comparison. Categorical

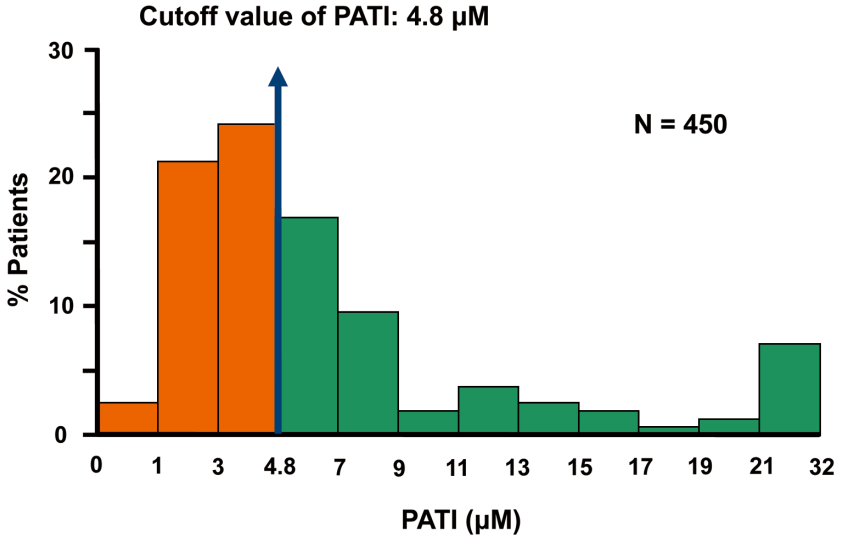

Figure 1. Distribution of the platelet aggregation threshold index (PATI) before stent implantation in 450 patients.

Table 1. Patients Characteristics

	High reactivity to ADP (n=232)	Low reactivity to ADP (n=218)	P value
Age (years)	72.1±9.5	70.1±10.2	0.02
Male (%)	68.5	68.4	0.96
Body mass index (kg/m^2)	24±3.4	23.8±3.0	0.64
Hypertension (%)	78.8	68.8	0.01
Diabetes mellitus (%)	44.4	41.3	0.50
Hyperlipidemia (%)	61.6	58.7	0.52
Current smoker (%)	11.6	10.1	0.59
Multi-vessel disease (%)	50.0	47.2	0.56
Previous myocardial infarction (%)	32.5	29.6	0.52
Calcium-channel blockers (%)	54.3	51.4	0.53
ACE inhibitors (%)	10.8	12.4	0.59
Angiotensin II receptor blockers (%)	48.3	49.1	0.86
β-blockers (%)	19.8	27.5	0.05
Statins (%)	57.3	50.5	0.14
Antidiabetic drug; insulin/oral/both (%)	6.5/28/2.6	2.8/27/1.8	0.22
Proton pump inhibitors (%)	22.3	23.6	0.79
Follow-up CAG (%)	74.9	73.9	0.80
Ejection fraction (%)	61.3±11.8	63.3±10.3	0.08

Values are mean or percent ± SD.
ADP, adenosine diphosphate; ACE, angiotensin-converting enzyme; CAG, coronary angiography.

variables are presented as numbers and percentage values, and were compared using the χ^2 test. The cumulative incidence of adverse events was estimated using the Kaplan-Meier method, and the curves were compared using the log-rank test. Multivariate analysis was used to identify the independent risk factors of adverse events. Confounding factors with P values ≤0.05 upon univariate analysis were subjected to multivariate logistic regression analysis. Confounding factors with continuous variables, as well as the PATI value predicting the composite primary endpoint after stenting at the maximum sensitivity, were determined as a cutoff value by receiver-operating characteristic (ROC) curve analyses. A P<0.05 was considered significant. All statistical analyses were undertaken using JPM for Windows version 5.1 (SAS Institute, Cary, NC, USA).

Results

Definition of High On-Treatment Platelet Reactivity to ADP

The cutoff value of the PATI that optimally predicted the composite primary endpoint within 1 year after DES implantation was determined using ROC analyses. If the cutoff value was set to PATI <4.8 μmol/L, the area under the curve of prediction of the composite primary endpoint during the 1-year period after DES implantation was 0.66 (P<0.001) and the sensitivity and specificity were 80% and 52%, respectively. PATI <4.8 μmol/L was defined as high on-treatment platelet reactivity to ADP.

Clinical Background of the Patients

The PATI values of the patients enrolled in the present study

Table 2. Baseline Biochemical Markers

	High reactivity to ADP (n=232)	Low reactivity to ADP (n=218)	P value
WBC (/μl)	6,141	6,242	0.53
Hb (g/dl)	13±1.8	13±1.7	0.27
Plt (×10⁴/μl)	20.9±5.1	21.2±5.8	0.55
AST (IU/L)	25.8±13.7	26.8±14.1	0.48
ALT (IU/L)	25.1±16.1	27.1±14.7	0.48
T-Cho (mg/dl)	199.5±94.7	188.7±30.6	0.43
HDL (mg/dl)	47±12	49±16	0.10
eGFR (ml·min^{-1}·1.73 m^{-2})	58.3±18.9	59.7±16.6	0.41
hs-CRP (mg/L)	3.01±5.6	2.45±4.9	0.35
Fibrinogen (mg/dl)	299.4±81.9	295.7±68.1	0.76
PATI (μmol/L)	2.8±1.1	12.1±8.6	<0.001

Values are mean±SD.
ADP, adenosine diphosphate; WBC, white blood cell; Hb, hemoglobin; Plt, platelet; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Cho, total cholesterol; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; PATI, platelet aggregation threshold index.

Table 3. Characteristics of the Procedure and Lesions

	High reactivity to ADP (n=335)	Low reactivity to ADP (n=313)	P value
Lesion type			
Type B2/C (%)	55	53	0.61
QCA			
Pre %DS (%)	68.4±13.5	67.3±13.8	0.27
Pre MLD (mm)	0.95±0.22	1.24±0.23	0.26
Pre RD (mm)	2.55±0.61	2.50±0.53	0.19
Lesion length (mm)	16.5±11.7	17.7±12.2	0.60
Post %DS (mm)	13.9±7.35	13.6±7.42	0.52
Post MLD (mm)	2.47±0.53	2.45±0.47	0.48
Rotational atherectomy (%)	10.5	9.0	0.50
SES/PES (%)	83/17	81/19	0.45
Stent diameter (mm)	2.92±0.39	2.89±0.41	0.36
Total stent length (mm)	24.2±14.8	22.7±14.8	0.16
Balloon pressure (atm)	17.8±3.36	17.7±3.75	0.60

Values are mean or percent±SD.
ADP, adenosine diphosphate; QCA, quantitative coronary angiography; %DS, percent diameter stenosis; MLD, minimal lumen diameter; RD, reference diameter; SES, sirolimus-eluting stent; PES, paclitaxel-eluting stent.

are shown in Figure 1. Patients were divided into 2 groups based on the cutoff value of PATI determined using the ROC curve. That is, patients with PATI <4.8 μmol/L and PATI ≥4.8 μmol/L were groups with high and low on-treatment reactivity to ADP, respectively. There were 232 and 218 patients in these groups, respectively. Their backgrounds are shown in Table 1. The prevalence of hypertension was high and age advanced in the high-reactivity group. There were no significant differences in blood test findings (Table 2), lesion type, or characteristics of the PCI procedure (Table 3) between the groups. PATI levels were not significantly different in patients who were and were not receiving proton-pump inhibitors (PPIs) (7.49±7.9 vs. 7.19±7.4 μmol/L, respectively, P=0.86).

Follow-up Coronary Angiography

Follow-up coronary angiography was undertaken in 74.9% (174/232) and 73.9% (160/218) of patients in the high- and low-reactivity groups, respectively (P=0.8). Upon comparison of 335 lesions in the 174 patients and 313 lesions in the 160 patients examined by follow-up coronary angiography in the high- and low-reactivity groups, respectively, the prevalence of restenosis was significantly higher in the high-reactivity group (17.9 vs. 6.4%, respectively, P<0.001).

Clinical Results

Table 4 shows the prevalence of cardiovascular events at 1-year follow up. The composite primary endpoint occurred in 55 patients (12.2%): 19.0% and 5.1% in the high- and low-reactivity groups, respectively, (P<0.001), and the events were mainly TLR (18.1% vs. 5.1%, respectively, P<0.001). Stent thrombosis occurred in 2 patients (0.86%) in the high-reactivity group, but in none of the subjects in the low-reactivity group, and this difference was not significant. Major hemorrhage occurred in 3 (1.29%) and 2 (0.92%) patients in the high- and low-reactivity groups, respectively, and this difference was not significant. No patient died of major hemorrhage (all 5 cases were intestinal hemorrhage) and no cerebral hemorrhage occurred. There was no significant difference in the prevalence of discontinuation within the 1-year follow-up period of DAPT between the groups. Ticlopidine was with-

Table 4. Clinical Outcome at 1-Year Follow up

	High reactivity to ADP (n=232)	Low reactivity to ADP (n=218)	P value
Composite primary endpoint, n (%)	44 (19.0)	11 (5.1)	<0.001
Cardiac death	3 (1.3)	0 (0)	0.92
Myocardial infarction	2 (0.86)	0 (0)	0.17
TLR	42 (18.1)	11 (5.1)	<0.001
Coronary artery bypass	1 (0.43)	0 (0)	0.33
Repeat PCI	41 (17.7)	11 (5.1)	<0.001
Urgent PCI due to ACS	5 (2.16)	0 (0)	0.11
Stent thrombosis	2 (0.86)	0 (0)	0.17
Major hemorrhage, n (%)	3 (1.29)	2 (0.92)	0.70
Discontinuation of DAPT, n (%)			
Within 6 months			
Aspirin	0 (0)	0 (0)	
Ticlopidine	6 (2.6)	5 (2.3)	0.84
Aspirin + Ticlopidine	0 (0)	0 (0)	
6–12 months			
Aspirin	0 (0)	0 (0)	
Ticlopidine	2 (0.86)	4 (1.83)	0.37
Aspirin + Ticlopidine	3 (1.29)	2 (0.92)	0.70

Values are the number of patients (%).
ADP, adenosine diphosphate; TLR, target lesion revascularization; PCI, percutaneous coronary intervention; ACS, acute coronary syndrome; DAPT, dual anti-platelet therapy.

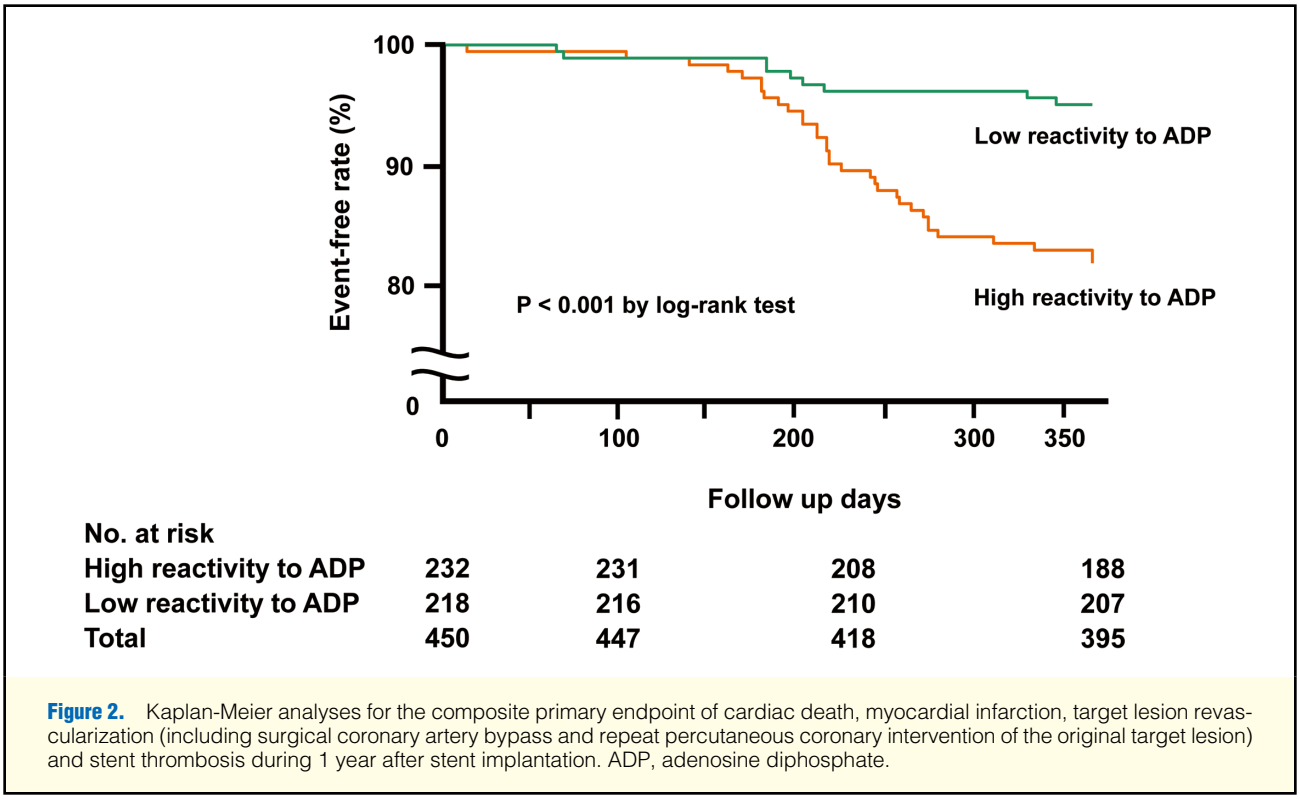

Figure 2. Kaplan-Meier analyses for the composite primary endpoint of cardiac death, myocardial infarction, target lesion revascularization (including surgical coronary artery bypass and repeat percutaneous coronary intervention of the original target lesion) and stent thrombosis during 1 year after stent implantation. ADP, adenosine diphosphate.

drawn within 6 months in 6 patients in the high-reactivity to ADP group (switched to clopidogrel in 3 subjects) and in 5 patients in the low-reactivity to ADP group (switched to clopidogrel in 2 subjects), but aspirin was not withdrawn in any patient. Between the 6th and 12th month, aspirin and ticlopidine as well as ticlopidine alone were withdrawn in 3 patients and 2 patients in the high-reactivity to ADP group, respectively, and in 2 patients and 4 patients (switched to clopidogrel in 1 subject) in the low-reactivity to ADP group, respectively. The cumulative incidence of the composite primary endpoint in the 1-year period after stenting is shown in Figure 2. Clinical and procedural characteristics according to the existence or not of

Table 5. Clinical and Procedural Characteristics According to the Existence or Not of a Composite Primary Endpoint

	Adverse ischemic events (+) (n=55)	Adverse ischemic events (–) (n=395)	P value
Age (years)	70.6±10.9	71.2±9.7	0.68
Male (%)	69	68	0.82
Body mass index (kg/m^2)	24.2±3.9	23.8±3.1	0.60
Hypertension (%)	71.7	74.3	0.68
Diabetes mellitus (%)	62.3	40.3	0.025
Hyperlipidemia (%)	60.3	60.2	0.98
Current smoker (%)	11.3	10.8	0.91
Multi-vessel disease (%)	50.9	48.3	0.72
Previous myocardial infarction (%)	36.4	30.6	0.56
Calcium-channel blockers (%)	56.6	52.4	0.56
ACE inhibitors (%)	15.1	11.1	0.41
Angiotensin II receptor blockers (%)	58.5	47.4	0.12
β-blockers (%)	26.4	23.2	0.61
Statins (%)	50.9	54.4	0.63
Antidiabetic drug; insulin/oral/both (%)	9.4/32.1/3.8	4.0/27.2/2.0	0.22
Proton pump inhibitors (%)	21	23	0.72
Ejection fraction (%)	61.1±11	62.4±11	0.46
Lesion type (B2+C) (%)	68.6	54.3	0.048
Pre %DS (>65%) (%)	67	50	0.013
Pre MLD (<0.85 mm) (%)	68	50	0.0096
Pre RD (<2.3 mm) (%)	53.8	34.1	0.006
Lesion length (>11 mm) (%)	78.8	55.8	0.001
Post %DS (>16%) (%)	51	35	0.15
Post MLD (<2.6 mm) (%)	83	66	0.0087
Rotational atherectomy (%)	15.1	9.1	0.19
SES/PES (%)	73/27	79/21	0.3
Stent diameter (>3.0 mm) (%)	49	56	0.32
Total stent length (>20 mm) (%)	71.7	42.1	<0.0001
Balloon pressure (>15 atm) (%)	81.1	75.3	0.34

Values are mean or percent ± SD.
Abbreviations see in Tables 1,3.

Table 6. Multivariate Logistic Regression Analysis for Predictors of Composite Primary Endpoints After Coronary Stenting

	OR	95%CI	P value
Diabetes mellitus	2.0	1.06–3.79	0.03
Lesion type (B2+C)	1.18	0.57–2.48	0.65
Pre %DS (>65%)	1.34	0.68–2.64	0.40
Pre MLD (<0.85 mm)	1.20	0.48–3.01	0.69
Pre RD (<2.3 mm)	1.85	0.92–3.73	0.85
Lesion length (>11 mm)	1.87	0.76–4.62	0.17
Post MLD (<2.6 mm)	1.61	0.68–3.80	0.27
Total stent length (>20 mm)	2.87	1.33–6.23	0.007
PATI <4.8 μmol/L	3.82	1.85–7.87	<0.001

OR, odds ratio; CI, confidence interval. Other abbreviations see in Tables 2,3.

adverse ischemic events are shown in Table 5. Upon multivariate analyses, high on-treatment reactivity to ADP, diabetes mellitus (DM), and the total length of the stent were independent predictors of cardiovascular events (Table 6).

Discussion

We investigated the association between ADP-induced platelet aggregation and ischemic as well as hemorrhagic events during a 1-year period in patients with stable angina pectoris who underwent coronary DES implantation. The prevalence of adverse ischemic events was significantly higher in the group with high on-treatment reactivity to ADP than in the group with low on-treatment reactivity to ADP, and this was due to the difference in the prevalence of TLR. No significant differences were noted in the prevalence of cardiovascular death, MI, or stent thrombosis between the groups, or in the prevalence of hemorrhagic events.

We evaluated ADP-induced platelet aggregation using the SFP method. The conventional method to measure platelet aggregation uses absorptiometry, in which platelet-rich plasma (PRP) is prepared by centrifugation, and its platelet aggregation reactions to a platelet-activating substance are monitored based on changes in absorbance.[18] However, PRP separation by centrifugation for routine clinical testing is complex and time-consuming. Even though the aggregation of platelets alone is often used, this method appears less likely to reflect platelet aggregation reactivity in a physiological setting. Therefore, a highly reproducible, rapid measurement method for aggregation using whole blood is necessary for routine clinical

practice. Measurement devices for whole-blood platelet aggregation used include Verify-Now® (Accumetrics, San Diego, CA, USA), and Multiplate® (Dynabyte, Medical, Munich, Germany).[3,19] We did not have data with regard to the correlation between ADP-induced platelet aggregation levels by the SFP method and those by these assay systems. However, the results obtained using the WBA-Neo Whole Blood Platelet Aggregation measurement device have been reported to be well correlated with those of the conventional method using absorptiometry and PRP.[20] Our results revealed that ADP-induced platelet aggregation reactivity after oral administration of thienopyridine varied widely among PCI patients. These results were in accordance with other studies.[5] This suggested that patients within our study had high on-treatment platelet reactivity to ADP.

With respect to setting the cutoff value of PATI at 4.8 μmol/L, patients with PATI <4.8 μmol/L were regarded as a group with high on-treatment platelet reactivity to ADP. Clinical adverse events after PCI were also predicted using ROC analyses in other reports, but ADP-induced platelet aggregation was measured using different methods.[21] The prevalence of cardiac events in the high-reactivity group was high, as previously reported.[1,4,9,22] In the present study, the difference between the groups was mainly due to the difference in the prevalence of TLR; the prevalence of cardiac death, coronary arterial bypass, MI, and stent thrombosis was similar between the groups. The present study was undertaken in a relatively low-risk population because we selected patients with stable angina pectoris and excluded acute-phase patients (such as those with AMI or unstable angina) and those undergoing hemodialysis. Also, the number of patients was low. These might have been reasons for the absence of significant differences in the prevalence of events other than TLR. The prevalence of stent thrombosis after DES implantation was reported to be 2.3% and 2.9% in the second and third year in European Bern/Rotterdam data, showing an increase of 0.6% per year,[23] whereas the prevalence in the j-Cypher registry (a large-scale observational study in Japan) was very low, that is, 0.34%, 0.54%, and 0.77% at 30 days, 1 year, and 2 years after stenting, respectively.[24] Thus, a large patient population is necessary to investigate the prevalence of stent thrombosis.

With respect to the risk factors of restenosis or TLR after DES implantation, the associations of DM,[25] hemodialysis,[26,11] stent length, and stent diameter[25] have been reported. A significantly high incidence of TLR in a clopidogrel low-responder group within 30 days after DES implantation has also been reported.[19] Upon multivariate analyses, in addition to DM and the total length of the stent, ADP-induced platelet aggregation reactivity before PCI was associated with the prognosis (in particular the prevalence of TLR) in the present study. Conversely, Schulz et al[27] reported that low responsiveness of platelets to clopidogrel did not have a significant impact on restenosis after DES at 1 year (TLR values: 10.9% in low responders vs. 9.5% in normal responders; binary angiographic restenosis: 13.9% vs. 15.9%, respectively). That study featured ≈33% of patients with acute coronary syndrome (ACS), a small number of patients treated with bare metal stents (BMS), and patients receiving the glycoprotein IIb/IIIa inhibitor, abciximab. They adopted the upper quintile of ADP-induced aggregation levels as the definition of clopidogrel low response instead of a cutoff value using a ROC curve analysis. The present study used ticlopidine for anti-platelet therapy instead of clopidogrel. Clopidogrel and ticlopidine are metabolized by CYP and have similar anti-platelet effects by blocking the P2Y12 platelet ADP receptor, but their pharmacokinetics and pharmacodynamics are different. Ticlopidine is reportedly a selective mechanism-based inhibitor of the P450 (CYP) 2C19.[28] The differences in results between these 2 studies might also have been affected by other drug-specific effects rather than the anti-platelet effects of clopidogrel. Clopidogrel has been reported to have anti-inflammatory effects after vascular injury in rabbits.[29] Although patients in the study by Schulz et al[27] received a loading dose of 600 mg clopidogrel, with a median time interval of >2 h before assessment of platelet function, the patients in the present study had taken ticlopidine for ≥1 week before intervention. Even if loading therapy of clopidogerl is undertaken, suppression of ADP-induced platelet aggregation might not have reached a peak. We believe that the most important factor related to the difference in the results of the 2 studies was the different duration of thienopyridine therapy after stent implantation (1 year in the present study compared with 6 months in the study by Schulz et al[27]). In an animal study, delayed wound healing was observed in neointimal lesions (fibrin deposition, protraction of inflammatory reactions, and incomplete coverage of the neointima) 28 days after DES implantation.[30] Kotani et al[31] reported that incomplete coverage of the neointima 3–6 months after DES implantation could be shown using coronary angioscopy. In a report in which the implantation site was observed 12 months after DES implantation using OCT, an organized thrombus was noted.[32] In patients treated with BMS, thrombus formation occurs in the early stages after stent implantation. However, in patients treated with DES, it might occur at a later phase after intervention compared with BMS due to delayed endothelial regeneration. We hypothesized that thrombotic tissue at the site of vascular injury might contain inflammatory cells secreting certain types of cytokines,[33] platelets releasing mediators that act as mitogens (eg, thromboxane A2, platelet-derived growth factor, serotonin),[34–36] and bone marrow-derived vascular progenitor cells, especially smooth muscle progenitor cells, which might differentiate into neointimal smooth muscle cells.[37] In addition, platelet deposition and subsequent thrombus formation at the site of stent implantation might play an important part in the formation of neointimal tissue. Also, an intra-stent organized thrombus transforming to neointimal tissue might be involved in the pathogenesis of restenosis after DES implantation. Therefore, P2Y12 platelet receptor inhibitors (anti-platelet thienopyridines such as ticlopidine and clopidogrel) powerfully suppressing platelet aggregation might be key players in restenosis after DES implantation. In fact, Evans et al[38] reported that P2Y12 platelet receptor-knockout mice showed 80% less neointimal formation after arterial injury compared with wild-type mice. On the basis of those results, fibrin adhesion around the stent and organization of mural thrombus due to delayed endothelial regeneration after DES implantation might have been the main causes of the restenotic tissue of the lesion. Restenotic lesions after DES implantation might be different from those after implantation of conventional BMS, and they might show a "thrombo-restenosis" pattern.[39] Therefore, longer thienopyridine therapy (eg, >6 months) might be necessary for the prevention of restenosis after DES implantaion compared with BMS implantation.

Regarding hemorrhagic events, Tsukahara et al[9] reported that the risk of hemorrhage was significantly increased in patients with a high PATI value, but no significant difference was noted in the prevalence of hemorrhagic events between groups in the present study. In a study by Tsukahara et al,[9] many patients with ACS and patients treated with oral ticlopidine or clopidogrel, were included. We limited the subjects to non-emergency PCI patients treated with oral ticlopidine, for whom oral administration of the anti-platelet drug might have

been initiated after evaluating the hemorrhagic risk.

Breet et al[40] undertook a prospective cohort study to compare the accuracy of 6 platelet function tests to identify the best test method for measurement of platelet activity during treatment. The results of 3 tests were associated with the incidence of atherothrombosis, but the prediction accuracy was only moderate. Platelet aggregation tests are markedly influenced by the timing of measurement after blood sampling, type and concentration of the aggregation-inducing substance, and measurement device. The significance and usefulness of a "point-of-care platelet function assay" are controversial. However, to increase the therapeutic effect and to avoid thrombotic or hemorrhagic complications, it might be necessary to standardize the evaluation of the pharmacological effects of thienopyridine anti-platelet drugs and accurately determine the effects in individual patients. Standardization of platelet function testing and accumulation of clinical data via a large-scale prospective study are required.

Study Limitations

First, the present study was carried out at a single center and had a small sample size. Therefore, larger-scale clinical studies are needed to further elucidate the risks of adverse cardiovascular events as well as bleeding associated with high on-treatment platelet reactivity to ADP in patients with DES implantation. Second, the prevalence of TLR (11.6%) was higher than that in a large-scale registry after SES implantation in Japan, the j-Cypher Registry (6.9%).[24] Relatively more patients with diffuse lesions accompanied by severe calcification treated with rotational atherectomy might have been included in the present study, thereby elevating the prevalence. The prevalence of TLR at 1 year after SES implantation after rotational atherectomy in consecutive patients including dialysis patients at our hospital was high (21.2%).[10] Sawada et al[41] stated that the overall prevalence of TLR after DES implantation in Japanese patients was 17%. Third, data were collected under oral administration of ticlopidine because we did not have permission to use clopidogrel in patients with stable angina on a health insurance system in Japan. Hence, a similar clinical study should be carried out under oral administration of clopidogrel in the future. Fourth, in the measurement of ADP-induced platelet aggregation using the SFP method, the PATI value showed a U-shaped variation depending on the timing of measurement after blood sampling, with the lowest value being at 1 h,[14] and similar results were obtained in a preliminary experiment done at our facility (data not shown). In the present study, blood was collected through an arterial sheath immediately before PCI in a Catheterization Suite, and measurement was after exactly 1 h. The cutoff value was 4.8 μmol/L, but it might vary depending on the blood sampling and measurement conditions at each facility. To establish a standard cutoff value for multiple facilities, it is necessary to undertake a multi-center clinical study with standardized blood sampling and measurement conditions. Finally, the ratio of patients with high on-treatment platelet reactivity to ADP in Japanese patients undergoing PCI might be higher than those reported in other countries.[8] A further study is necessary to investigate if the high ratio is a characteristic of Japanese patients.

Conclusion

Although the present study included several limitations, we found that high on-treatment platelet reactivity to ADP was associated with adverse cardiac events, (mainly TLR) after DES implantation in patients with stable angina pectoris by measuring ADP-induced platelet aggregation before PCI using the SFP method. The present study warrants further investigations involving a large number of patients to confirm that the high on-treatment platelet reactivity to ADP is a predictor of TLR after DES implantation in patients with stable angina.

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