Restenosis had been the major drawback of PCI using bare-metal stent. Extremely high rate of repeated revascularization procedures in this study might be largely attributable to expanded use of PCI for more complex subsets of patients and high rate of angiographic follow-up in the Japanese clinical practice. However, the advent of drug-eluting stents have already markedly ameliorated the problems related to restenosis in real world clinical practice.[19]

There are several important limitations of this study. As compared with prior observational studies,[8,9] the sample size was not large enough to detect small differences in survival rates between the CABG and PCI groups. Although the definition of elderly patients was prespecified and seems clinically reasonable, the cut-off value of 75 years of age is arbitrary. Although variations in the frequencies of some anatomic factors such as numbers of diseased vessels, involvement of proximal LAD, and presence of total occlusions were adjusted for comparative analyses, our conclusions might not be applicable to those patients with other anatomic complexities, such as diffuse disease, heavy calcification or bifurcation that were not evaluated in this study. Furthermore, important medications, statins in particular, to prevent cardiovascular events are obviously underused. More optimal use of medications might have changed the long-term outcome of both CABG and PCI. Finally, the baseline characteristics such as age, body mass index, and prevalence of diabetes in the current population were markedly different from prior studies.[8,9] Although it is beyond the scope of the current article to discuss on the contribution of these demographic features to the different outcome in comparison to prior studies, we should admit that differences in racial, cultural, and socioeconomic factors might hinder generalization of the conclusions of this study outside Japan. Relatively low rate of recurrent coronary events in the Japanese population demonstrated in the REACH registry might have favorable influence on survival outcome after PCI.[20]

Despite the abovementioned study limitations, we would conclude that for patients with multivessel coronary artery disease, survival outcomes were similar among those who underwent either CABG or PCI with bare-metal stents in real-world clinical practice in Japan, when elderly patients are excluded from analysis.

Appendix

List of Clinical Research Coordinators

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Disclosures

None.

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Letter to the Editor

Effects of ICD implantation on quality-adjusted life years in patients with congestive heart failure ☆

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Abstract

Background: Effects of prophylactic implantable cardioverter–defibrillator (ICD) on quality-adjusted life years (QALYs) in patients with congestive heart failure are uncertain.
Methods: We developed a decision model for patients at risk of sudden death due to reduced ejection fraction and who had no history of life-threatening ventricular arrhythmias. It estimated the QALYs for ICD strategy as a primary prevention for sudden cardiac death and conventional strategy without antiarrhythmic therapies.
Results: In a 3-year time period, the QALYs for patients with conventional strategy were higher than that of ICD strategy (2.19 years vs. 2.14 years). When the mortality rate of conventional strategy exceeded 8.6%/year and the hazard ratio of death for the ICD strategy was lower than 0.70, the ICD strategy was the superior treatment option.
Conclusions: The QALYs of patients with ICD could be lower than that of conventional strategy. Incorporating quality of life could affect decision making of ICD implantation.

Keywords: Decision analysis; Implantable cardioverter–defibrillator; Primary prevention; Quality-adjusted life years; Reduced left ventricular ejection fraction

1. Introduction

Congestive heart failure (CHF) is a major and growing public health burden in the world and the mortality rate remains high, despite the current progress in medical management [1]. Sudden death accounts for approximately one third of the mortality, and a significant number of these sudden deaths are due to ventricular arrhythmias. A prophylactic implantable cardioverter–defibrillator (ICD) can decrease the mortality of CHF patients with reduced left ventricular ejection fraction (LVEF) [2]. However, ICD implantation may also be associated with deterioration in quality of life (QOL) because of repeated cardioversion shocks and device-related complications [3,4]. Therefore, we conducted a decision analysis to evaluate the effects of prophylactic ICD implantation for CHF patients with reduced LVEF on quality-adjusted life years (QALYs).

2. Methods

We developed a decision model for CHF patients with reduced LVEF and no history of life-threatening ventricular arrhythmias, which was similar to the published decision model for cost-effectiveness analysis of ICD [5]. We compared ICD strategy and conventional strategy without antiarrhythmic therapies as a primary prevention for sudden cardiac death. Because previous studies followed patients with prophylactic ICD for up to 4 years [6,7], we set the time

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frame for 3 years. We adjusted life expectancy for QOL by using health state utilities, which represent patient preference for a given health state and are ranged from 0 to 1. Zero represents death and 1 represents perfect health without any physical or mental discomfort [8]. QALYs were calculated by multiplying year in a given health state by the utility value for that health state. We used the decision model to estimate QALYs for each strategy, taking into account discomfort caused by disease itself or any complication, and the magnitude of the risk of death. All analyses were performed with TreeAge Pro 2005 Suite (TreeAge Software, Inc., Williamstown, MA).

Estimation of the clinical parameters was based on the literature [6,9]. We calculated the transition probabilities from one state to another within 1 cycle (1 year) by the exponential conversion method [10]. Because data on utility of patients with prophylactic ICD implantation were not available, we obtained the 4 utility values about ICD strategy with and without complication, and conventional strategy with and without complication from 27 cardiologists by the time trade-off method [8]. The mean values were used for a base case analysis. The ranges for the sensitivity analyses were determined on the basis of the 97.5% confidence intervals (CIs). Parameter estimates are summarized in Table 1. We conducted extensive sensitivity analyses because of the wide variation of probabilities for clinical outcomes and utilities for individual patients.

3. Results

Among patients with ICD strategy, 93.4%, 87.1% and 81.3% survived after 1, 2 and 3 years, respectively. Among patients with conventional strategy, 91.4%, 83.5% and 76.2% survived after 1, 2 and 3 years, respectively. This result was consistent with the reported survival probabilities [6]. We calculated the expected survival years for 3 cycles and found that it was 2.71 years for ICD strategy and 2.63 years for conventional strategy. When adjusted for QOL, the longer expected QALYs were 2.19 years for conventional strategy, followed by 2.14 years for ICD strategy.

One-way sensitivity analyses for the top 4 variables with marked effects on QALYs are shown in Fig. 1. If the utility for well patients with conventional strategy was lower than 0.816 or if the utility for well patients with ICD strategy exceeded 0.808, ICD strategy became the most preferable strategy. When the two clinically important variables in daily practice (mortality of conventional strategy and the hazard ratio of death with ICD strategy) were subjected to two-way sensitivity analysis, ICD strategy became superior strategy in case of that the mortality of conventional strategy exceeded 8.6%/year and hazard ratio of death in ICD strategy was lower than 0.70.

4. Discussion

Our analysis sheds light on an important issue regarding prophylactic ICD implantation for CHF patients with reduced LVEF. Although longer survival years could be expected by ICD implantation, utility of patients with ICD might be lower than that of patients with conventional strategy. As a result, the QALYs for patients with ICD strategy could be lower than those for patients with conventional strategy.

Anxiety brought about by repeated shocks could account for lower utilities of patients with ICD. Sporadic shocks was reported an important factor of reduced QOL in patients with ICD [3,4]. In addition to the true ICD shocks for fatal arrhythmias, about 10 to 20% of patients with an ICD experienced inappropriate ICD shocks [6,7]. Patients who had survived life-threatening arrhythmias would easily accept ICD, but the fear for life-threatening arrhythmias could be smaller in patients who had not experienced such life-threatening arrhythmias but had to receive an ICD as a primary prevention. Namerow et al. suggested that patients who received prophylactic ICD might be more influenced by the negative aspects of ICD implantation than patients who received ICD for secondary prevention [4]. Thus, incorporating utility of individual patients into decision of prophylactic ICD implantation appeared important and could affect decision making of treatment strategy.

Table 1
Parameters estimates for clinical outcomes and utilities in the decision model

Parameter		Base-case estimate (range)	Reference(s)
Probability	Operative death in ICD strategy	0 (0–0.003)	[3,6]
	Complications in ICD strategy at the first year	0.158 (0.130 to 0.187)	[6,9]
	Complications in ICD strategy at the second and third year	0.114 (0.089 to 0.139)	[6,9]
	Withdrawal from ICD strategy per year	0.011 (0.003 to 0.019)	[6]
	Complications in conventional strategy per year	0.092 (0.074 to 0.110)	[9]
	Death in conventional strategy per year	0.086 (0.064 to 0.108)	[6]
Hazard ratio of death	ICD vs. conventional strategy	0.77 (0.62 to 0.96)	[6]
Utility	ICD strategy with well condition	0.792 (0.670 to 0.915)	questionnaire
	ICD strategy with any complication	0.589 (0.447 to 0.731)	
	Conventional strategy with well condition	0.833 (0.737 to 0.929)	
	Conventional strategy with any complication	0.604 (0.458 to 0.749)	

ICD: implantable cardioverter–defibrillator.

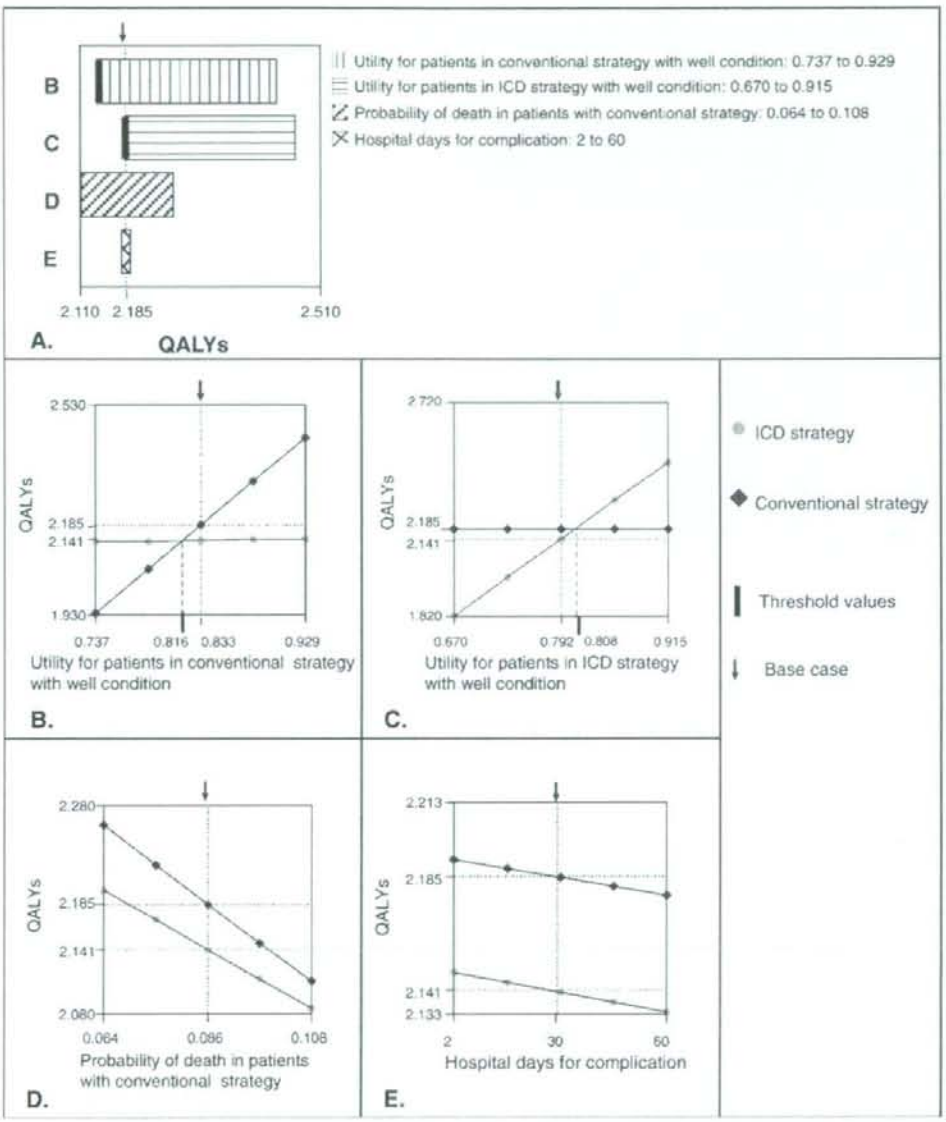

Fig. 1. Top 4 variables with marked effects on quality-adjusted life years. Panel A: the top 4 variables and the range of quality-adjusted life years (QALYs). Panel B: if the utility for patients in conventional strategy with well condition was lower than 0.816, implantable cardioverter–defibrillator (ICD) strategy became the treatment of choice. Panel C: if the utility for patients in ICD strategy with well condition exceeded 0.808, ICD strategy became the treatment of choice. Panel D: probability of death in patients with conventional strategy had marked effects on QALYs, but it did not change the treatment of choice. Panel E: hospital days for complication had some effects on QALYs, but it did not change the treatment of choice.

Our study had several limitations inherent to the study design. First, utility values were elicited from a survey of cardiologists. This group differs from typical recipients of prophylactic ICDs, but previous studies to assess QOL in patients with heart failure reported that the mean utility values were 0.65–0.77 [11,12], and these values were similar to those elicited in our study. Second, we did not take into account patients with combined amiodarone and ICD treatment. In actual practice, antiarrhythmic drugs have been commonly used in conjunction with ICD. The effect of such concomitant use of amiodarone with an ICD on QALYs should be explored by future studies.

Because prophylactic ICD for CHF patients with reduced LVEF prolongs life but does not improve symptoms, QOL could be as an important issue as the length of life for these patients. Our study focused on this unavoidable and important issue. Further studies are required to assess the utilities of actual CHF patients with reduced LVEF facing prophylactic ICD implantation.

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Better Survival With Statin Administration After Revascularization Therapy in Japanese Patients With Coronary Artery Disease

— Perspectives From the CREDO-Kyoto Registry —

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Background The importance of statins in cardiovascular prevention has been demonstrated in various patient subsets. This study aimed to evaluate the effects of statins on long-term outcomes of Japanese patients undergoing their first coronary revascularization.

Methods and Results A total of 9,225 patients undergoing their first coronary revascularizations during 2000–2002 were divided into 2 groups according to the use of statins at discharge; patients with acute myocardial infarction were not included. Statins was administered to only 28.5% (n=2,630) of the patients. The median follow-up period was 3.5 years. Patients on statin therapy showed lower all-cause (5.2% vs 10.0%; p<0.0001) and cardiovascular (3.2% vs 6.2%; p<0.0001) mortality than those without statins (n=6,595) by Kaplan-Meier analysis and log-rank test. After adjustment by multivariate analysis according to 29 variables, statin therapy remained as an independent predictor of reduced all-cause (relative risk ratio (RR) 0.71, 95% confidence interval (CI) 0.59–0.86, p=0.0005) and cardiovascular (RR 0.72, 95% CI 0.56–0.91, p=0.0067) mortality. The validity of RR of statin therapy in multivariate analysis was further confirmed by risk adjustment using propensity scores (all-cause mortality: propensity-adjusted RR 0.70, 95% CI 0.58–0.85, p=0.0003; cardiovascular mortality: propensity-adjusted RR 0.70, 95% CI 0.54–0.89, p=0.0038).

Conclusions Statin therapy started at hospital discharge was associated with increased chance of survival in Japanese patients undergoing their first coronary revascularization. (*Circ J* 2008; **72:** 1937–1945)

Key Words: Coronary artery disease; Mortality; Revascularization; Statins

Optimized medical therapy, as well as appropriate lifestyle modification, is important to reduce cardiovascular risks in the secondary prevention of coronary artery disease (CAD). Common medications for cardiovascular prevention in contemporary clinical practice include antiplatelet drugs, HMG-CoA reductase inhibitors (statins), inhibitors of the rennin–angiotensin system (ie, angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II type I receptor blockers (ARB)), and β-adrenergic blockers[1,2] In particular, consistent prognostic benefits of statins have been shown in a number of primary as well as secondary prevention trials[3–8] The study subjects have been widely distributed from hypercholesterolemic patients without CAD to specific high-risk groups such as diabetic patients, patients with acute coronary syndrome and patients undergoing percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG)[9–13] Cardiovascular events can be further prevented when the low-density lipoprotein-cholesterol level is intensively decreased below the normal range with statin therapy[11,14] Much of this evidence has been obtained from randomized trials in the United States or Europe where the prevalence and mortality of CAD are higher and higher doses of statins are approved by public medical insurance systems, relative to Japan. Therefore, the data may not be directly applicable to practice in different clinical and genetic back grounds such as Japanese patients. Recently, coronary risk reduction by pravastatin has been shown in Japanese patients in the setting of primary prevention[15] In addition, recent trials have demonstrated effective cardiovascular prevention by

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Investigators in the Coronary REvascularization Demonstrating Outcome study in Kyoto (CREDO-Kyoto) registry are listed in Appendix 1.

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Table 1 Baseline Characteristics of the Patients According to Statin Treatment at Hospital Discharge

Characteristic	Statin-treated (n=2,630)	Statin-non-treated (n=6,595)	p value
Demographic characteristics			
Age (mean±SD)	65.2±10.0	67.8±9.9	<0.0001
≥75 years (%)	18.1	26.4	<0.0001
Male gender (%)	62.6	73.7	<0.0001
Mode of revascularization PCI (%)	79.8	66.3	<0.0001
BMI (kg/m^2)	24.4±3.2	23.4±3.2	<0.0001
≥25 kg/m^2 (%)	39.4	27.8	<0.0001
Family history of CAD (%)	19.1	14.4	<0.0001
Current smoking (%)	27.9	28.5	NS
Coexisting conditions			
Unstable angina (%)	7.7	7.5	NS
Prior MI (%)	22.6	26.5	0.0001
History of CHF (%)	11.8	18.4	<0.0001
History of CVA (%)	13.6	17.4	<0.0001
Peripheral vascular disease (%)	6.5	7.6	NS
Atrial fibrillation (%)	4.8	7.5	<0.0001
Anemia: Hb <10 g/dl (%)	3.9	7.8	<0.0001
COPD (%)	1.2	2.9	<0.0001
Liver cirrhosis (%)	1.6	3.7	<0.0001
Serum TC	211.9±44.4	193.1±35.3	<0.0001
≥220 mg/dl (%)	39.5	21.0	<0.0001
Serum LDL-C	130.1±41.1	119.0±31.2	<0.0001
≥130 mg/dl (%)	46.6	34.2	<0.0001
Serum HDL-C	50.2±14.8	47.3±13.5	<0.0001
<40 mg/dl (male); <50 mg/dl (female) (%)	32.8	37.8	<0.0001
Serum TG	163.6±103.3	137.2±84.2	<0.0001
≥150 mg/dl (%)	46.2	30.9	<0.0001
Hypertension (%)	71.3	68.4	0.0059
Diabetes mellitus (%)	40.6	38.0	0.0235
Insulin Tx (%)*	19.5	22.5	0.0495
CKD: GFR <60 ml/min (%)	31.8	42.9	<0.0001
Left main CAD (%)	7.3	10.3	<0.0001
Proximal LAD lesion (%)	41.1	42.6	NS
Multivessel disease (%)	63.6	63.6	NS

*Values are presented as frequencies (%) within diabetic patients.

PCI, percutaneous coronary intervention; BMI, body mass index; CAD, coronary artery disease; NS, not statistically significant; MI, myocardial infarction; CHF, congestive heart failure; CVA, cerebrovascular accident; Hb, hemoglobin; COPD, chronic obstructive pulmonary disease; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; Tx, therapy; CKD, chronic kidney disease; GFR, glomerular filtration rate; LAD, left anterior descending.

statin therapy in Japanese patients with acute myocardial infarction (AMI)[16,17] The association of statin therapy with better outcomes has also been shown in Japanese patients with AMI or angiographically proven CAD[18,19]

The aim of this study was to assess the preventive effects of medical therapies prescribed at hospital discharge on the long-term prognosis in Japanese CAD patients undergoing their first coronary revascularization by PCI or CABG, with an emphasis on statins. The patients have been registered from 30 institutions cooperating in the Coronary REvascularization Demonstrating Outcome study in Kyoto (CREDO-Kyoto) registry.

Methods

This study was approved by the institutional review boards or ethics committees of all participating institutions. Because the study subjects were retrospectively enrolled, written informed consent was not obtained, in concordance with the guidelines for epidemiologic studies issued by the Ministry of Health, Labor and Welfare of Japan. However, 73 patients were excluded because of their refusal to participate in the study when contacted for follow-up.

CREDO-Kyoto Registry and the Study Subjects

Consecutive patients who underwent their first coronary revascularization during 2000–2002 have been enrolled in the CREDO-Kyoto registry. Patients with AMI within 1 week after onset have not been included. Thirty institutions (Appendix 1) participated in the multicenter registry, and the baseline and follow up data for 9,877 patients have been obtained[20] After excluding patients with malignant diseases (n=496), those who died in hospital (n=62) and those without precise information about their medical treatment at discharge or follow-up data (n=94), 9,225 patients were subjected to the analyses.

Data Collection, Definition and Follow-up

Clinical and analytical information of the study patients were collected from hospital charts or databases in each center by independent clinical research coordinators (Appendix 2) according to predetermined definitions. The baseline information of the patients included age, sex, smoking habit, body mass index (BMI), mode of revascularization, biochemistry before revascularization procedure, and comorbidities and background conditions such as hypertension, diabetes mellitus (DM), chronic kidney disease (CKD), anemia, peripheral vascular disease, history of heart failure, prior myocardial infarction (MI) or cerebrovascular acci-

Table 2 Medications at Hospital Discharge

Medication	All patients (n=9,225)	Statin-treated (n=2,630)	Statin-non-treated (n=6,595)	p value
Statins (%)	28.5	100	0	<0.0001
ACEI or ARB (%)	32.9	38.9	30.6	<0.0001
ACEI (%)	20.4	24.5	18.7	<0.0001
ARB (%)	13.4	15.6	12.5	<0.0001
β-adrenergic blocker (%)	16.6	22.6	14.2	<0.0001
Calcium-channel blockers (%)	59.4	60.2	59.0	NS
Nitrates (%)	62.3	63.2	61.9	NS
Antiplatelet medications (%)	96.3	97.3	95.9	0.0014
Aspirin (%)	88.3	89.4	87.9	0.0382
Ticlopidine (%)	56.5	62.6	54.0	<0.0001

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker. Other abbreviation see in Table 1.

dent. CKD was regarded as present when the glomerular filtration rate estimated by Cockcloft-Gould formula was <60 ml/min. Anemia was defined as blood hemoglobin level <10 g/dl. Peripheral vascular disease was as present when carotid, aortic and/or other peripheral vascular disease was being treated or was scheduled for surgical or endovascular interventions. Angiographic data of CAD, as well as precise information of the medical treatment at hospital discharge, were also obtained. The patients were followed up with respect to mortality for a median of 3.5 years. An independent clinical events committee adjudicated the events. All deaths were confirmed by medical records or telephone interview of the patients' families, and death was regarded as cardiovascular in origin unless obvious noncardiovascular causes could be identified. MI was adjudicated according to the definition in the Arterial Revascularization Therapy Study[21] Within 1 week of the index procedure, only Q-wave MI was adjudicated as MI. Stroke during follow-up was defined as symptomatic stroke.

Statistical Analysis

All continuous variables are expressed as means ± SD. Statistical significance of differences in subject baseline demographics between the patients with statin therapy and the patients without statin therapy at hospital discharge were assessed by a Student t-test for parametrically distributed continuous variables, the Wilcoxon signed-ranks test for nonparametrically distributed continuous variables or a Pearson's χ^2 test for categorized data analyses.

Following the descriptive statistics, we used Kaplan–Meier estimates to plot the percentage of patients in each group free from any death, cardiovascular death, MI, stroke or any revascularization procedure. The log-rank test was used to identify significant differences in unadjusted survival rates. To determine the significant and independent prognostic factors for mortality, we listed 22 potential baseline variables: mode of revascularization, old-old age (≥75 years), male gender, BMI (≥25 kg/m^2), current smoking, hypertension, DM, peripheral vascular disease, cerebrovascular disease, atrial fibrillation, chronic obstructive pulmonary disease, CKD, liver cirrhosis, anemia, unstable angina, prior MI, history of congestive heart failure, high serum total cholesterol (TC ≥220 mg/dl) or triglycerides (≥150 mg/dl) level, left main coronary artery (LMCA) disease, proximal left anterior descending artery lesion and multivessel disease, and 7 potential risk-reducing pharmacotherapies at hospital discharge: statins, ACEI, ARB, β-adrenergic blockers, antiplatelet drugs, nitrates and calcium-channel blockers. Thus, all continuous variables were dichotomized for fitting proportional assumption according to the predetermined clinical contexts. The relationships between the 29 variables and each of the endpoints were assessed using a multivariate Cox proportional hazards model with stepwise procedure. Variable selection was carried out, stepping up with $p<0.05$ as the requirement for inclusion and stepping down by deleting the term with the highest p-value until all remaining terms were significant with p-values<0.05. In the multivariate analyses assessing the relationships between the 29 variables and MI, stroke and revascularization as the end-

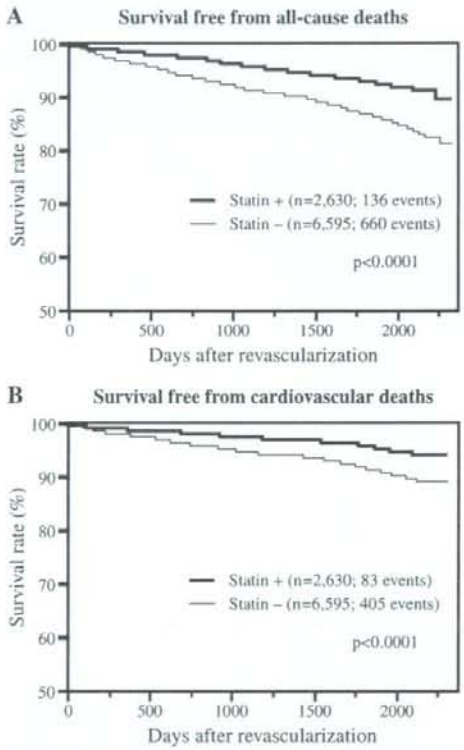

Fig 1. Kaplan–Meier analysis of cumulative rates of survival in coronary artery disease patients undergoing their first revascularization with vs without statin therapy at hospital discharge for all-cause (A) and cardiovascular (B) mortality.

Table 3 Multivariate Analysis for Prognostic Factors of All-Cause Mortality

Parameter	*RR*	*95%CI*	*p value*
Antiplatelet drugs at discharge	*0.61*	*0.46–0.80*	*0.0003*
BMI 25 kg/m²	*0.69*	*0.57–0.84*	*0.0002*
Statins at discharge	*0.71*	*0.59–0.86*	*0.0005*
Nitrates at discharge	*0.86*	*0.74–1.00*	*0.0446*
CVA	*1.25*	*1.06–1.47*	*0.0075*
Diabetes mellitus	*1.28*	*1.11–1.48*	*0.0009*
Male gender	*1.32*	*1.12–1.55*	*0.0007*
Revascularization by PCI	*1.35*	*1.14–1.59*	*0.0004*
Multivessel disease	*1.40*	*1.19–1.66*	*<0.0001*
Liver cirrhosis	*1.65*	*1.21–2.23*	*0.0014*
COPD	*1.68*	*1.23–2.29*	*0.0010*
Peripheral vascular disease	*1.70*	*1.38–2.08*	*<0.0001*
Age ≥75 years	*1.93*	*1.65–2.25*	*<0.0001*
History of heart failure	*2.10*	*1.80–2.45*	*<0.0001*
CKD	*2.12*	*1.78–2.52*	*<0.0001*
Hb <10 g/dl	*2.28*	*1.89–2.74*	*<0.0001*

RR, relative risk ratio; CI, confidence interval. Other abbreviations see in Table 1.

Table 4 Multivariate Analysis for Prognostic Factors of Cardiovascular Mortality

Parameter	*RR*	*95%CI*	*p value*
Antiplatelet drugs at discharge	*0.63*	*0.45–0.90*	*0.0102*
Statins at discharge	*0.72*	*0.56–0.91*	*0.0067*
BMI ≥25 kg/m²	*0.76*	*0.60–0.98*	*0.0335*
Male gender	*1.26*	*1.03–1.54*	*0.0220*
Revascularization by PCI	*1.29*	*1.06–1.58*	*0.0118*
Diabetes mellitus	*1.30*	*1.08–1.56*	*0.0064*
CVA	*1.37*	*1.12–1.68*	*0.0026*
Multivessel disease	*1.51*	*1.21–1.88*	*0.0003*
Age ≥75 years	*1.62*	*1.33–2.00*	*<0.0001*
Peripheral vascular disease	*1.67*	*1.28–2.16*	*0.0001*
History of heart failure	*2.35*	*1.94–2.86*	*<0.0001*
Hb <10 g/dl	*2.42*	*1.93–3.03*	*<0.0001*
CKD	*2.94*	*2.32–3.71*	*<0.0001*

Abbreviations see in Tables 1,3.

points, statin therapy was included in the variables, irrespective of its significance and independence as a prognostic predictor.

To evaluate the consistency of the association of statin therapy with reduced all-cause and cardiovascular mortality, multivariate analyses using the Cox proportional hazards models were also performed in predetermined subgroups. These included patients ≥75 years/<75 years, male/female, PCI/CABG, diabetic/non-diabetic, hypertensive/non-hypertensive patients, and patients with/without high serum TC, high serum triglyceride, CKD, history of heart failure or prior MI. The same factors used for analysis of the total cohort were incorporated in the multivariate models for subgroup analyses. Interaction analyses were also carried out between each categorized subgroup.

Because standard adjustment measures were not always valid, because of suboptimal assessment of degree of overlapping baseline characteristics, propensity score analysis was also performed as described and implemented by Rubin and others, using the baseline characteristics as potential prognostic factors.[22–24] Essentially, this involves calculation of a propensity score, which is the probability that any given individual patient would be part of the statin-treated group as opposed to the statin-non-treated group. The prognostic factors to calculate the propensity score were the same 29 factors included as variables of the multivariate analyses.

All analyses were performed using SAS Ver 9.1.3 (SAS Institute Inc, Cary, NC, USA) and all reported p-values are 2-sided.

Results

Subject Demographics and Characteristics of Medical Therapy at Discharge

A total of 9,225 study patients were divided into 2 groups based on the use of statin therapy at hospital discharge: 28.5% (n=2,630) of the subjects were on statin therapy and 71.5% (n=6,595) were not. Because of the large number of study patients and the observational study design, statistically significant differences were observed in many variables at baseline between the 2 groups. Significantly different variables include demographic characteristics such as the proportion of patients ≥75 years, male gender and mode of revascularization, comorbidities such as history of heart failure, other atherosclerotic diseases, dyslipidemia, diabetes and CKD, and the existence of LMCA disease (Table 1).

Prescription rates for ACEI, ARB, ACEI/ARB and β-adrenergic blockers at discharge were 20.4%, 13.4%, 32.9% and 16.6%, respectively. Most patients (96.3%) were treated with antiplatelet medications. Nitrates (62.3%) and calcium-channel blockers (59.4%) were prescribed in a considerably high proportion of the subjects (Table 2).

Table 5 RR of Statin Therapy for All-Cause and Cardiovascular Mortality by Propensity Score Analysis Compared With Multivariate Analysis

Endpoints and analysis	*RR*	*95% CI*	*p value*
All-cause mortality			
Multivariate analysis	*0.71*	*0.59–0.86*	*0.0005*
Adjustment by propensity score analysis			
Quintiles category	*0.70*	*0.58–0.85*	*0.0003*
Propensity score	*0.73*	*0.61–0.89*	*<0.0001*
Stratification of quintiles category	*0.70*	*0.58–0.85*	*0.0003*
Cardiovascular mortality			
Multivariate analysis	*0.72*	*0.56–0.91*	*0.0067*
Adjustment by propensity score analysis			
Quintiles category	*0.70*	*0.54–0.89*	*0.0038*
Propensity score	*0.73*	*0.57–0.93*	*0.0102*
Stratification of quintiles category	*0.69*	*0.54–0.89*	*0.0036*

Abbreviations see in Table 3.

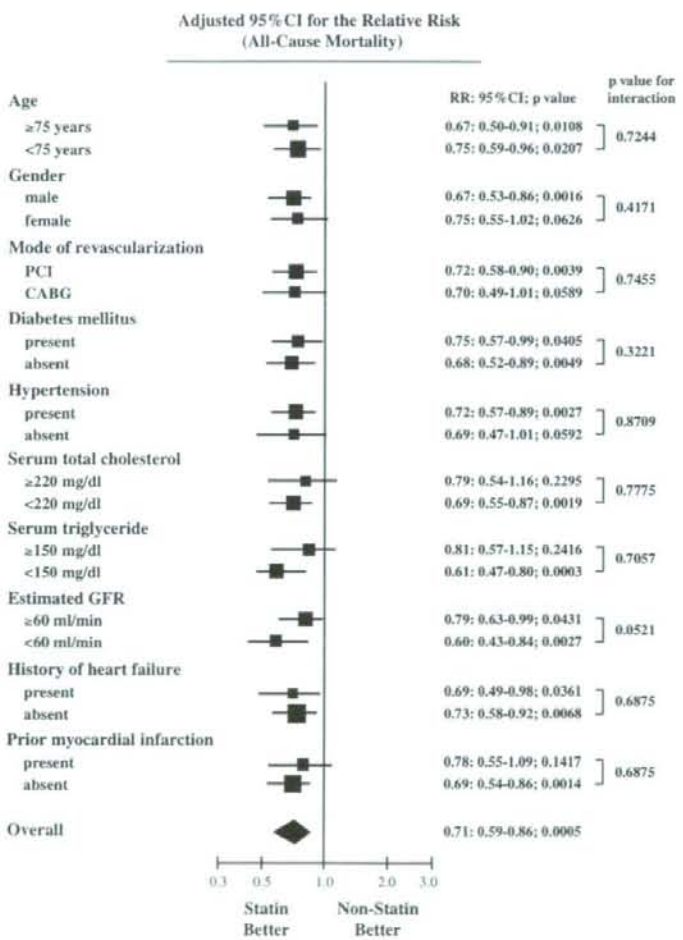

Fig 2. Relative risk ratio (RR) and 95% confidence interval (CI) for all-cause mortality for patients with vs without statin therapy in various patient subgroups. CABG, coronary artery bypass graft surgery; PCI, percutaneous coronary intervention.

Association of Statin Therapy With Unadjusted Survival

During the follow-up (median=3.5 years) with 96% follow-up rate at ≥2 years, 136 deaths (5.2%) and 660 deaths (10.0%) occurred in the statin-treated and the statin-non-treated groups, respectively. Among them, 83 deaths (3.2%) in the statin-treated and 405 deaths (6.2%) in the statin-non-treated groups were from cardiovascular events. Kaplan–Meier survival method and log-rank analysis in the 2 groups indicated that the unadjusted chance of survival free from all-cause, as well as cardiovascular, deaths was significant-

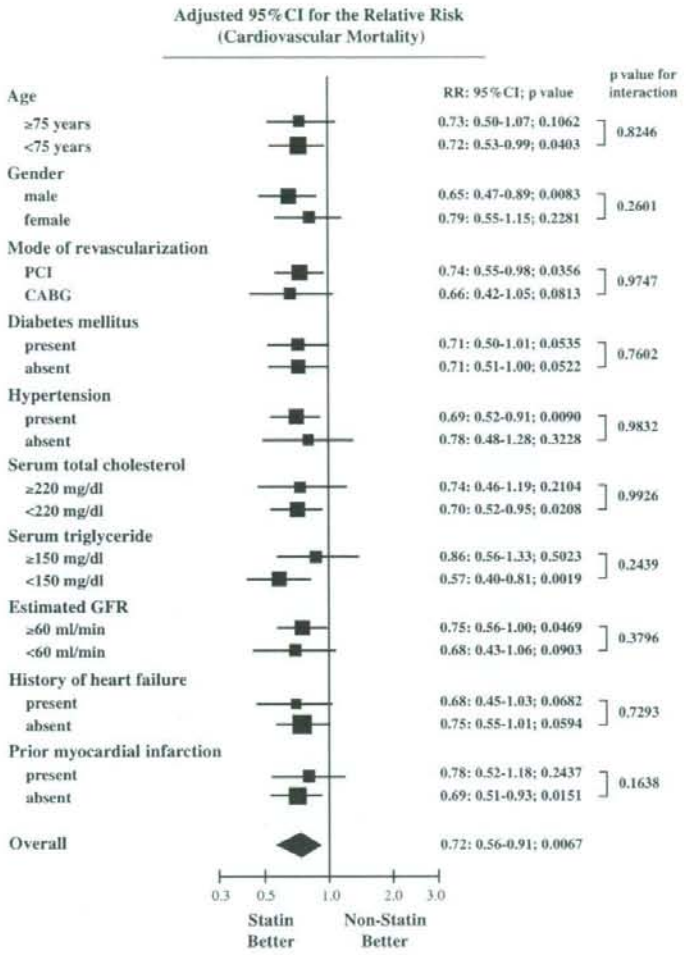

Fig 3. Relative risk ratio (RR) and 95% confidence interval (CI) for cardiovascular mortality for patients with vs without statin therapy in various patient subgroups. CABG, coronary artery bypass graft surgery; PCI, percutaneous coronary intervention.

ly greater in the statin-treated group (p<0.0001, Fig 1).

Association of Statin Therapy With Adjusted Survival

The results of the multivariate analysis of the predictors of all-cause mortality using stepwise procedure are shown in Table 3, where the relative risk ratio (RR), 95% confidence interval (CI) and p-value of each factor are given. Statin therapy at discharge remained an independent predictor of an increased chance of survival (RR 0.71, 95% CI 0.59–0.86, p=0.0005). Antiplatelets (RR 0.61, 95% CI 0.46–0.80, p=0.0003) and higher BMI (RR 0.69, 95% CI 0.57–0.84, p=0.0002) were also included as independent factors predicting increased chance of survival. Nitrates might marginally contribute to the survival of patients.

Table 4 shows the results of the multivariate analysis of predictors of cardiovascular mortality. Similar to the results of the analysis of all-cause mortality, statin therapy at discharge was an independent predictor of survival free from cardiovascular death.

The RR of statin therapy for all-cause (RR 0.70, 95% CI 0.58–0.85, p=0.0003) or cardiovascular (RR 0.70, 95% CI 0.54–0.89, p=0.0038) mortality adjusted by quintiles category of propensity score was quite similar to the result of multivariate analysis (Table 5). In the sensitivity analysis, the RR of statin therapy adjusted by propensity score as a numeric variable and the RR of statin therapy adjusted by stratification of quintiles category of propensity score also showed similar results (Table 5). Thus, the validity of the RR of statin therapy for all-cause or cardiovascular mortality in the multivariate analysis was confirmed by risk adjustment using propensity scores.

Consistency of Better Survival in Patients With Statin Therapy

Subgroup analyses indicated consistently lower all-cause, as well as cardiovascular, mortality in patients with statin therapy in all subgroups (Figs 2,3). Point-estimate of the relative risk was less than 1.0 in all subgroups and no significant interaction was found between the subgroups in each category. The relative risk for all-cause mortality was statistically significantly lower in the statin-treated group than in the statin-non-treated group in patients at ages <75 years and

≥75 years, patients treated by PCI, male patients, both diabetic/non-diabetic patients, hypertensive patients, patients with serum TC <220 mg/dl or serum triglyceride <150 mg/dl, patients with/without CKD, patients with/without heart failure, and patients without prior MI (Fig 2). The significant association of statin therapy and the lower risk for cardiovascular mortality was also seen in various subgroups (Fig 3). Despite the consistency of the association of statin therapy and better outcomes, statins were used significantly less frequently in subgroups at higher cardiovascular risk such as male patients (25.3% on statins), older patients (21.5%), patients with CKD (22.9%), patients with a history of heart failure (20.3%) or a prior MI (25.4%). Post-CABG patients were significantly less frequently prescribed statins at hospital discharge (19.3%) than post-PCI patients (32.4%).

Association of Statin Therapy With Future MI, Stroke and Coronary Revascularization

Although crude survival free from stroke was significantly better in patients with statins, by Kaplan–Meier analysis and by log-rank test (p=0.0013), statin therapy did not remain as an independent prognostic factor of stroke (444 total events: 99 in the statin-treated group, 345 in the statin-non-treated group; RR 0.83, 95% CI 0.66–1.04, p=0.0999) after adjustment by multivariate analysis. With regard to MI (257 total events: 69 in the statin-treated group, 188 in the statin-non-treated group; RR 0.89, 95% CI 0.67–1.17, p=0.4019) and any revascularization (2,835 total events: 876 in the statin-treated group, 1959 in the statin-non-treated group; RR 0.96, 95% CI 0.88–1.04, p=0.2768), both crude survival and the results of the multivariate analyses failed to prove the significant association of statin therapy with better outcomes.

Discussion

In the present study, we have shown that statin therapy at hospital discharge is associated with better outcomes in Japanese patients after their first coronary revascularization. All-cause, as well as cardiovascular, mortality was significantly lower in patients with statin therapy than in those without statins, after adjustments for coexisting coronary risk factors, mode of revascularization and concomitant medical treatments. The association of statin therapy with better survival was consistently shown among various patient subgroups.

Characteristics of the Clinical Background of the Study Patients

Our study patients consisted of a secondary prevention cohort with established CAD undergoing their first PCI or CABG. Approximately 70% of the patients underwent PCI and comprised a unique group at high risk of cardiovascular events associated with stent deployment, such as stent thrombosis and restenosis, and hemorrhagic complications related to dual antiplatelet drug therapy. Moreover, the subjects' baseline characteristics indicated a high prevalence of multiple coronary risk factors, suggesting the need for intensive risk factor management. Although racial differences in susceptibility to CAD might exist and previous observational studies have shown a lower prevalence of CAD in Japan relative to the United States,[25,26] the all-cause mortality of the present patients without statins (10% at 3.5 years of median follow-up) appeared comparable with that of the control group in the 4S study (12% at 5.4 years of median follow-up), a secondary prevention study in patients with stable CAD in Europe.[3] Thus, the present study has been performed in a unique Asian patient group at a mortality risk as high as a Caucasian secondary prevention cohort.

Better Survival in Patients With Statin Therapy and its Consistency

All-cause, as well as cardiovascular, mortality was significantly lower in Japanese patients with statin therapy who underwent their first coronary revascularization by PCI with bare-metal stent or by CABG. The findings are consistent with a previous small randomized trial[12] and an observational study that was carried out in Europe in patients after PCI.[27] Subgroup analysis revealed a survival advantage in patients with statin therapy in various high-risk subgroups such as older patients and patients with CKD. Analysis in patients with TC ≥220 mg/dl did not indicate a significant difference in all-cause or cardiovascular mortality between the statin-treated and the non-treated groups. This unexpected result might be related to the smaller number of patients in the group with TC ≥220 mg/dl (26.4% of all subjects). Indeed, there was a trend to favor statin therapy in regard to relative risk for all-cause or cardiovascular mortality, and the RR of the statin-treated group for cardiovascular mortality was comparable between patients with TC ≥220 mg/dl and those with TC <220 mg/dl. Thus, the results added further evidence to the consistent benefits of statins for secondary cardiovascular prevention. Because an admission for a coronary revascularization procedure is a good opportunity to optimize medical therapy for CAD, most patients undergoing PCI or CABG should be considered for the indication of statins.[28]

The multivariate analyses failed to show significant differences in the prevalence of MI, stroke and any coronary revascularization between the statin-treated and non-treated groups, although the relative risk appeared to favor statin therapy in the analyses for MI and stroke. Because the majority of the repeated revascularization procedures were performed within 1 year of the first revascularization, because of restenosis after stenting, the preventive role statins for restenosis appears deniable. In regard to MI and stroke, the prevalence might not be sufficiently high to show differences (2.8% for MI and 4.8% for stroke). In particular, longer follow-up or analysis in a larger patient population could increase the number of events and show a significant differences between the statin-treated and the non-treated groups in the prevalence of stroke, because a clear tendency of fewer strokes in the statin-treated group was observed. Another possible explanation may be the effect of statins on the severity of MI and stroke. It has been reported that pretreatment with statins decreases myocardial injury or periprocedural mortality during PCI or CABG.[29–31] Fonarow et al have shown in a large scale observational study that new or continued statin therapy in the first 24 h of the index AMI was associated with lower mortality compared with no statin use, but not with the incidence of recurrent MI.[32] Moreover, an association of prior statin use with smaller infarct size or better outcomes has been reported in patients with ischemic stroke.[33,34] Thus, it is possible that statin therapy is associated with reduced mortality by its limiting of tissue damage during ischemic events and subsequent invasive therapeutic procedures for the events.

Characteristics of the Medical Therapy of the Study Patients

Despite the better survival of statin-treated patients, the present study also indicated suboptimal use of statins in Japanese high-risk patients during the study period. The use of ACEI, ARB and β-adrenergic blockers at hospital discharge was also less frequent than we expected from the data for the US and Europe[27,35] The reported lower prevalence of CAD in Japan relative to the United States might cause underuse of preventive medical therapies[25,26] However, the prevalence of CAD in the general population cannot explain the future risk of patients with established CAD. Because the patients analyzed in this study were a secondary prevention cohort with CAD, the use of statins in Japan was apparently suboptimal in 2000–2002.

The use of nitrates or calcium-channel blockers was much more frequent than in the reports from Europe analyzing patients after coronary revascularization[7] This result agrees with a recent study of Japanese CAD patients[19] Higher prevalence of coronary vasospastic angina in Japanese than in Caucasians may partly explain this difference[36] Thus, our study patients had unique concomitant medical treatments in comparison with previous reports from Western countries[7] and the results of the present study enable assessment of the consistency of the beneficial effects of statins in patient groups with distinctive backgrounds and concomitant medical therapy.

Strengths and Limitations

The present study was based on data from the CREDO-Kyoto registry, a multicenter registry of 30 hospitals in Japan. The number of study patients was sufficient to assess the impact of each prognostic factor on mortality and the size of each participating hospital is variable. The data have been collected by trained clinical research coordinators based on a detailed manual and the follow-up rate was 96% at ≥2 years. Thus, the sample size, distribution of the size of the participating hospitals and the method of data acquisition strengthen the reliability of the data, and the results represent the "real world" clinical practice for CAD patients undergoing revascularization during 2000–2002 in Japan.

Several limitations that are common to all observational studies should be noted in the interpretation of the results. First, there were significant differences between the statin-treated and the statin-non-treated groups in many of the patients' baseline characteristics. Second, the statins and their doses were inconsistent and it is difficult to assess the efficacy of each statin at a particular dose, although the efficacies of statins may not be identical. Finally, the information about medical therapy was obtained at only 1 time point, hospital discharge, for each patient. Therefore, the adherence of the patients to the medications and the cross-over of patients between the statin-treated and the statin-non-treated groups have not been considered in the analyses. The lipid profiles of the patients before statin therapy are also uncertain, and the cholesterol levels without statin therapy might have been higher in the statin-treated group. Thus, the possibility that the results have been influenced by these factors cannot be eliminated.

Conclusion

In the present study of Japanese patients undergoing their first coronary revascularization therapy, starting medical treatment with statins by hospital discharge was associated with lower all-cause as well as cardiovascular mortality. The results can be seen as a rationale for the comprehensive use of statins for secondary prevention in patients with CAD.

Acknowledgements

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Appendix 1

List of Participating Centers and Investigators

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Appendix 2

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Circ J 2008; **72:** 1844–1851

Evaluation of the Antiplatelet Effects of Cilostazol, a Phosphodiesterase 3 Inhibitor, by VASP Phosphorylation and Platelet Aggregation

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Background Cilostazol, a phosphodiesterase 3 inhibitor, is an antiplatelet drug that is widely used for preventing cardiovascular events, although, to date, there are few methods for evaluating its effects.
Methods and Results Blood samples were taken at baseline and at 3 and 12 h in 10 healthy male subjects after 100 mg cilostazol intake. Each sample was examined by Western blot for phosphorylation levels of vasodilator-stimulated phosphoprotein (VASP), an abundant cAMP-dependent kinase substrate in platelets, and by the optical aggregometer for ADP- and collagen-induced aggregation, before and after 8 nmol/L prostaglandin E1 (PGE_1) treatment. Cilostazol intake did not affect VASP phosphorylation levels or the maximal aggregation rates without PGE_1 treatment. However, cilostazol intake apparently enhanced PGE_1-induced VASP phosphorylation and PGE_1-mediated reduction of ADP- and collagen-induced maximal aggregation rates. Levels of VASP phosphorylated at Ser157 were correlated and the maximal aggregation rates induced by ADP were inversely correlated with cilostazol concentrations in the plasma.
Conclusion The antiplatelet effects of cilostazol intake could be evaluated by measuring VASP phosphorylation levels and maximal aggregation rates in platelets by ex vivo treatment with a low concentration of PGE_1. (*Circ J* 2008; **72:** 1844–1851)

Key Words: Antiplatelet; Cilostazol; Platelets; Prostaglandins; Signal transduction

Because of its antiplatelet and vasodilating effects[1,2] cilostazol is widely used in clinical practice for prevention of stent thrombosis and stent restenosis[3–6] It also reduces the risk of stroke by approximately 40%[7] and prevents recurrence of cerebral infarction[8] Furthermore, cilostazol has been recommended in the international guideline, TASCII[9,10] as a first-line therapy for peripheral arterial disease because it improves the symptoms[11]

Some agonists, including prostacyclin, a well established antiplatelet agent[12] and adenosine[13] increase cAMP via *Gαs*-coupled receptors. A synthetic compound, prostaglandin E1 (PGE_1) also increases cAMP levels in platelets via the prostacyclin receptor[14] and is used in the clinical setting for its antiplatelet and vasodilating effects[15] Thus, increased cAMP levels inhibit platelet activation. Cilostazol, a phosphodiesterase 3 inhibitor, blocks cAMP degradation and is thus expected to have an antiplatelet function.

Vasodilator-stimulated phosphoprotein (VASP), which is a regulator of actin-cytoskeletal reorganization, is abundantly expressed in platelets[16] and plays an important role in their aggregation[17] VASP is phosphorylated at serine 157 (Ser157) and serine 239 (Ser239) by both cAMP-dependent protein kinase (A-kinase) and cGMP-dependent protein kinase (G-kinase). Therefore, VASP phosphorylation levels are considered to reflect levels of cAMP and cGMP in platelets[18,19] Recently, VASP phosphorylation levels were used to monitor the effects of *Gαi*-coupled $P2Y_{12}$ ADP receptor inhibitors (ie, thienopyridine derivatives such as ticlopidine and clopidogrel)[20] because these drugs inhibit the reduction of cAMP levels induced by ADP in platelets.

Although the effectiveness of antiplatelet drugs such as aspirin and thienopyridine have been evaluated, evaluation of cilostazol's effectiveness is limited to a few reports[21,22] because there are few methods available for monitoring its antiplatelet effect. In this report, we show that the effects of cilostazol intake can be monitored by analyzing the VASP phosphorylation levels and the ADP- and collagen-induced maximal aggregation rates (MARs) of platelets under PGE_1 treatment. Because VASP phosphorylation can not be detected without PGE_1 stimulation, our data suggest that the function of cilostazol is to enhance the platelet-inactivating cAMP signals downstream of *Gαs*-coupled receptors stimulated by agonists such as prostacyclin and adenosine.

Methods

Cilostazol Intake and Platelet-Rich Plasma (PRP) Preparation

This study was approved by the Ethics Committee, Faculty of Medicine, Kyoto University. Ten healthy male

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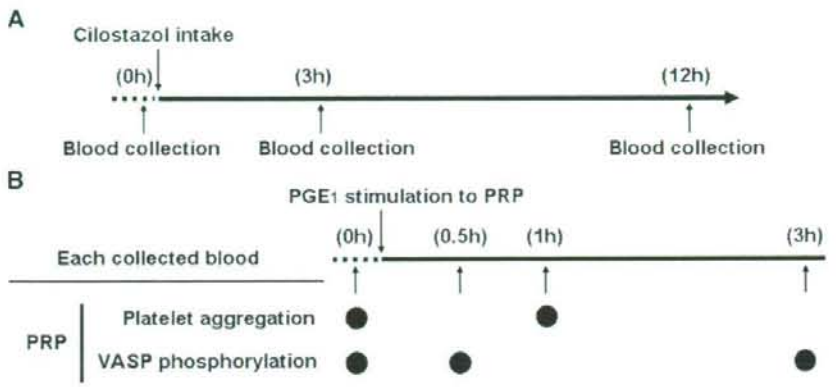

Fig 1. Study protocols for (A) blood sampling after cilostazol intake and (B) analysis of platelet aggregation and vasodilator-stimulated phosphoprotein (VASP) phosphorylation after blood sampling. At the 12-h time point post-cilostazol intake, only VASP phosphorylation was examined. PGE_1, prostaglandin E_1; PRP, platelet-rich plasma.

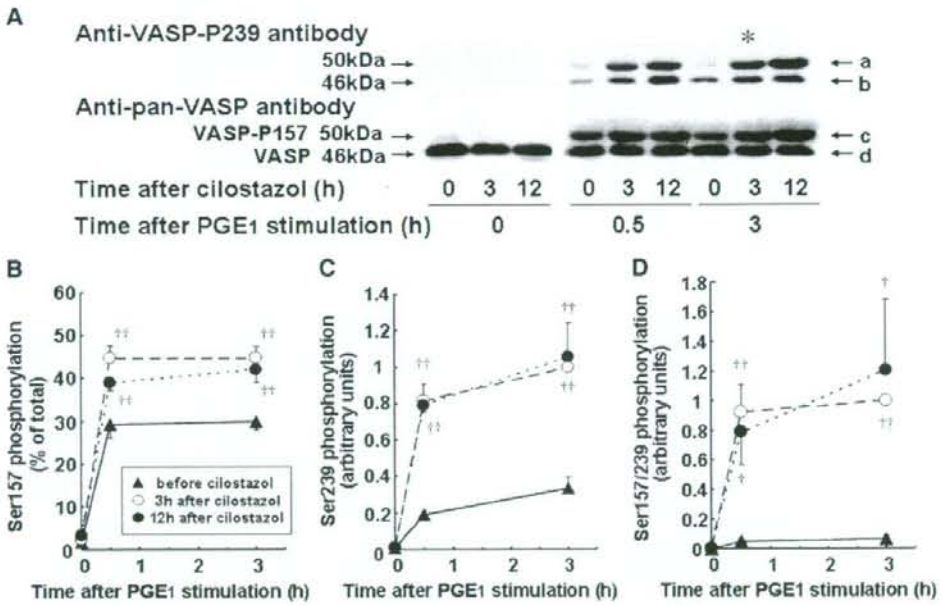

Fig 2. Effects of cilostazol intake on vasodilator-stimulated phosphoprotein (VASP) phosphorylation of platelets under prostaglandin E_1 (PGE_1) treatment. (A) VASP phosphorylation in platelets at baseline, and at 3 and 12 h after cilostazol intake, were examined under PGE_1 treatment for 0.5 and 3 h by immunoblotting with anti-pan-VASP and anti-VASP-P239 antibodies as described in the Methods. (B–D) Quantification of phosphorylated VASP shown in (A) (n=10) using Image J 1.33u software (NIH). The signals in the "a", "b", "c" and "d" bands in (A) are VASP-P157/239, VASP-P239, VASP-P157, and non-phosphorylated VASP, respectively, as described in the Methods. (B) VASP-P157 levels expressed as c/(c+d)× 100% are shown. (C) The level of phosphorylation of Ser239 was quantified by measuring a+b. The signal of a+b (* in Fig 2A) was defined as the standard (1.0) and other signals were expressed as ratios of the standard. (D) The level of VASP-P157/239 was quantified by measuring "a". The signal of "a" (* in Fig 2A) was defined as the standard (1.0) and other signals were expressed as ratios of the standard. $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$ vs before cilostazol. The data are mean±SE (n=10).

physicians, aged 32–48 years old, from Kyoto University Hospital, who understood the significance of this investigation and possible adverse side-effects of cilostazol, voluntarily participated in this study, and gave written informed consent. Subjects were not permitted to take any concomitant medication for 7 days prior to and during the study period. Each subject took a single dose of 100 mg cilostazol (Otsuka Pharmaceutical Co Ltd, Tokushima, Japan) after breakfast. A blood sample, containing $1.79–2.53\times10^5$ ($2.31\pm0.23\times10^5$, mean±SE) platelets/μl, was collected at baseline

Table 1 Clinical and Biochemical Characteristics of the Study Subjects

Age, years	*36.8±6.12*
RBC×10¹²/L	*5.04±0.18*
Hemoglobin×10² g/L	*1.55±0.04*
WBC×10⁹/L	*5.84±0.78*
Platelets×10¹¹/L	*2.31±0.27*
Fibrinogen, g/L	*2.29±0.22*
PT (INR)	*1.05±0.06*
aPTT, s	*32.2±3.11*
Fasting glucose, g/L	*0.94±0.07*
HbA1c, %	*4.74±0.24*
AST, IU/L	*19.7±2.5*
ALT, IU/L	*20.2±5.26*
Gamma GTP, IU/L	*33.1±24.7*
Total cholesterol, g/L	*2.04±0.21*
HDL-cholesterol, g/L	*0.51±0.09*
Triglyceride, g/L	*1.18±0.63*
CK, IU/L	*112±32*
UA, mg/L	*58.4±8.11*
BUN, mg/L	*131±25.3*
Creatinine, mg/L	*7.92±0.69*
Na, mmol/L	*140±2.12*
K, mmol/L	*4.2±0.14*

Data are mean±SE; n=10 males.
RBC, erythrocyte; WBC, leukocyte; PT (INR), prothrombin time (international normalized ratio); aPTT, activated partial thromboplastin time; HbA1c, hemoglobin A1c; AST, aspartate aminotransferase; ALT, alanine aminotransferase; gamma GTP, gamma glutamyl transpeptidase; HDL, high-density lipoprotein; CK, creatine kinase; UA, uric acid; BUN, blood urea nitrogen.

and at 3 and 12 h after cilostazol intake (Fig 1), using a 21G needle with tourniquet, into a glass tube containing sodium citrate at a final concentration of 0.313%. PRP was prepared by centrifugation of the blood sample at 200 g at 25°C for 10 min, and platelet-poor plasma was prepared by centrifugation at 2,000 g at 25°C for 10 min.

Analysis of VASP Phosphorylation

The PRPs prepared from blood samples taken at baseline, and at 3 and 12 h after 100 mg cilostazol intake, were treated with or without PGE1 (Sigma, St Louis, MO, USA) at 8 nmol/L for 0.5 and 3 h at 30°C. Platelets were collected by centrifugation at 700 g at 4°C for 5 min and added to sodium dodecylsulfate (SDS)-containing Laemmli buffer,[23] followed by incubation at 95°C for 10 min. The samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotted with anti-pan-VASP (M4, Alexis, San Diego, CA, USA) and anti-phospho (239)-VASP (16C2, Nano Tools Antikörpertecnik, Tiningen, Germany) mouse monoclonal antibodies as primary antibodies and horseradish peroxidase-conjugated anti-mouse IgG polyclonal sheep antibody (GE Healthcare) as the secondary antibody, followed by visualization with ECL chemiluminescent material (GE Healthcare). A typical result is shown in Fig 2A. It has been reported that VASP phosphorylated at Ser157 (VASP-P157) migrates to a 50 kD position in SDS-PAGE analysis, whereas VASP phosphorylated at Ser239 (VASP-P239) migrates to a 46 kD position similar to the non-phosphorylated form.[24] Therefore, the "a", "b", "c" and "d" bands shown in Fig 2A were VASP phosphorylated at both Ser157 and Ser239 (VASP-P157/239), VASP-P239, VASP-P157 irrespective of phosphorylation at Ser239, and non-phosphorylated VASP, respectively. These bands were quantified using Image J 1.33u software (NIH). VASP-P157 levels were expressed as c/(c+d)×100%. The level of VASP-P239 was quantified by measuring a+b. The a+b level at 3 h post-cilostazol intake with PGE1 treatment for 3 h (* in Fig 2A) was defined as the standard (1.0) and other signals were expressed as ratios of the standard. The level of VASP-P157/239 was quantified by measuring "a". The "a" level at 3 h post-cilostazol intake, with PGE1 treatment for 3 h (* in Fig 2A), was defined as the standard (1.0) and other signals were expressed as ratios of the standard.

Analysis of Aggregation of PRP

The PRPs, with or without 8 nmol/L PGE1 treatment for 1 h at 30°C, prepared from blood samples taken at baseline and at 3 h after 100 mg cilostazol intake were individually stimulated by 5 and 20 μmol/L ADP (Sigma) and 1 μg/ml collagen (Horm, Germany) at 30°C, and the aggregations were analyzed under stirring using a light transmission aggregometer, MCM HEMA TRACER 313 (MC Medical, Tokyo, Japan), where the degree of light transmission of PRP was defined as 0% of the aggregation rate, and the cognitive platelet-poor plasma as 100%.[25] The MAR of platelets and the rate of inhibition of platelet aggregation (IPA) were used for data analysis. IPA was calculated using the following formula: IPA (%)=[(MAR0–MAR3)/MAR0]×100, where MAR0 is the MAR at baseline and MAR3 is the MAR at 3 h after cilostazol intake.

Laboratory Testing and Analysis of the Concentration of Cilostazol

General serum values were measured by the SRL Laboratory (Tokyo, Japan). Plasma concentrations of cilostazol at baseline and at 3 and 12 h after 100 mg cilostazol intake were measured by BML, Inc (Tokyo, Japan).

Statistical Analyses

Data are expressed as mean±SE (n=10). Paired t-test was used for comparison of continuous variables over time. Correlation between cilostazol plasma concentrations and IPA (%) was assessed by Spearman's rank test. Correlation between cilostazol plasma concentrations and VASP-P157 levels was assessed by longitudinal analysis methods because the cilostazol concentrations changed over time. We used the PROC MIXED command of SAS, with serine 157 phosphorylation of VASP of platelets as a dependent variable, and cilostazol concentration and time as independent variables. Correlation between cilostazol plasma concentrations and MARs was assessed in the same way. As for VASP-P157, the linearity of time was evaluated by the Log likelihood method. If the linearity of time was appropriate, then longitudinal analysis was conducted with the time as linear. Otherwise, we conducted it using the profile method. We used SAS software version 9.1 (SAS Institute Inc, Cary, NC, USA); p values less than 0.05 were defined as significant.

Results

Effects of Cilostazol Intake on Platelet Aggregation and VASP Phosphorylation

Baseline laboratory parameter values were within normal limits for all subjects (Table 1). It has been shown that, following administration, the maximum plasma concentration of cilostazol is reached in approximately 3 h, and that it has a half-life of approximately 12 h.[26] Therefore, we analyzed platelet functions at baseline and at 3 and 12 h after 100 mg cilostazol intake. Compared with the baseline data, no reduc-

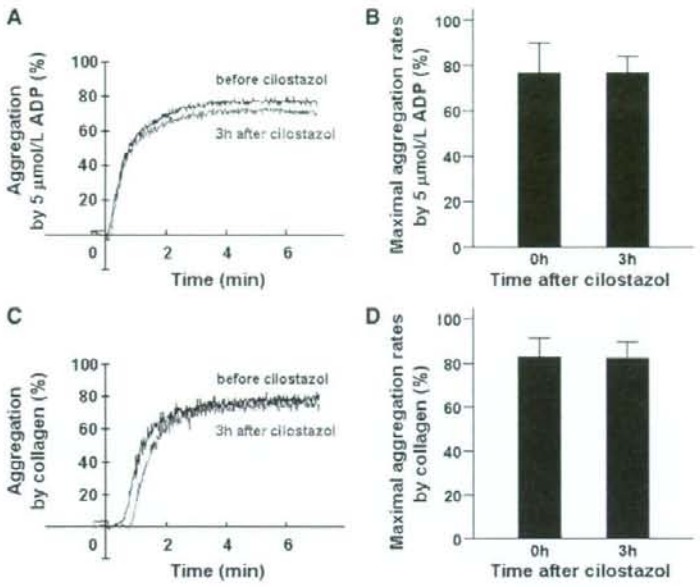

Fig 3. Effects of cilostazol intake on aggregation of platelets without prostaglandin E1 treatment. (A–D) Platelets at baseline and at 3 h after cilostazol intake were examined for 5 μmol/L ADP-induced (A,B) and 1 μg/ml collagen-induced (C,D) aggregation. Representative results of the aggregation curve (A,C), and the maximal aggregation rates expressed as mean±SE (n=10) (B,D) are shown.

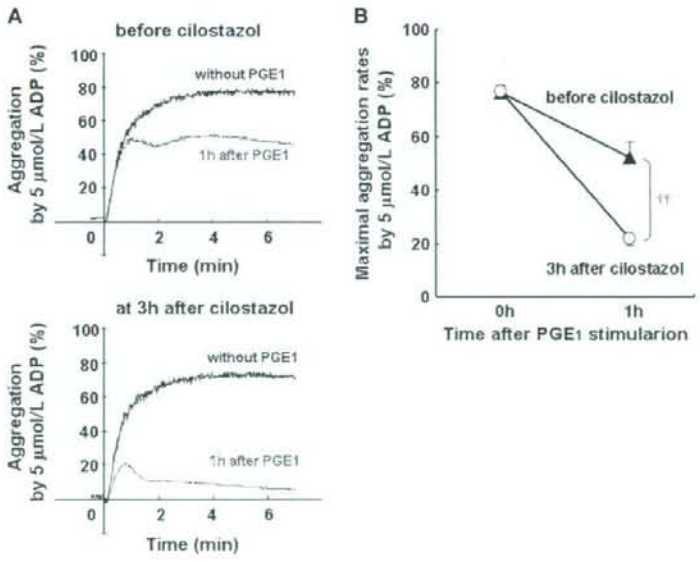

Fig 4. Effects of cilostazol intake on ADP-induced platelet aggregation under prostaglandin E1 (PGE1) treatment. (A) Representative curves of 5 μmol/L ADP-induced aggregation of platelets at baseline and at 3 h after cilostazol intake, with or without 8 nmol/L PGE1 treatment for 1 h. (B) Quantification of the maximal aggregation rates of the data shown in (A). At baseline (▲) and at 3 h after cilostazol (○). The data are mean±SE (n=10). ††$p<0.01$ vs before cilostazol.

tion was detected in ADP- or collagen-induced aggregation of platelets after cilostazol intake (Figs 3A–D). Because cilostazol is an inhibitor of phosphodiesterase 3, which degrades cAMP, it would be expected to cause an increase in the cAMP level in platelets, and also to raise phosphorylated VASP levels through the activation of A-kinase. As shown in Fig 2A, however, phosphorylated VASP was not detected in the platelets prepared from samples collected at baseline, or at 3 or 12 h post-cilostazol intake. Thus, cilostazol failed to increase cAMP to a level adequate enough to increase VASP phosphorylation levels or to reduce platelet aggregability induced by collagen or ADP.

Cilostazol Enhanced VASP Phosphorylation Levels Induced by PGE1

Although cilostazol intake did not induce VASP phosphorylation, we considered that it might have an effect on VASP phosphorylation, if cAMP was produced in response to stimuli. Therefore, VASP phosphorylation in isolated platelets under treatment of 8 nmol/L PGE1 was analyzed by Western blot with anti-pan-VASP and anti-phospho (239)-VASP antibodies. We evaluated VASP, VASP-P157, VASP-

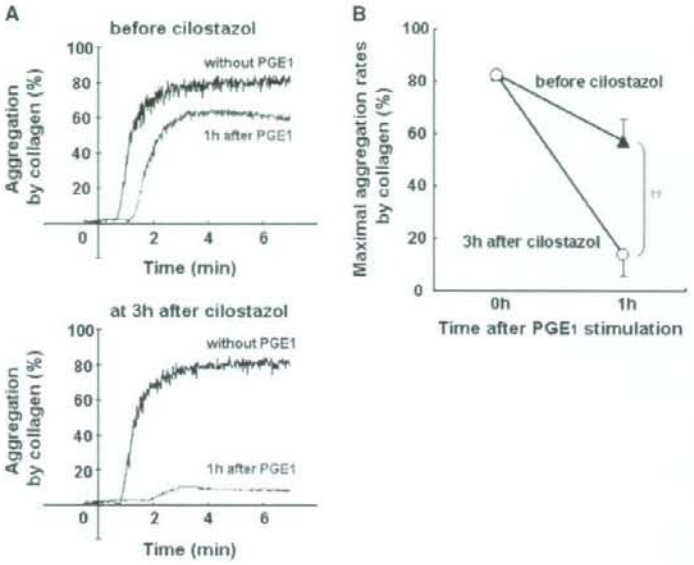

Fig 5. Effects of cilostazol intake on collagen-induced platelet aggregation under prostaglandin E1 (PGE1) treatment. (A) Representative curves of 1 μg/ml collagen-induced aggregation of platelets at baseline and at 3 h after cilostazol intake, with or without 8 nmol/L PGE1 treatment for 1 h. (B) Quantification of the maximal aggregation rates of the data shown in (A). At baseline (▲) and at 3 h after cilostazol (○). The data are mean± SE (n=10). ††p<0.01 vs before cilostazol.

P239 and VASP-P157/239 individually (see Methods). Treatment with PGE1 induced VASP phosphorylation at Ser157 and Ser239 in a time-dependent manner (Fig 2). Although VASP-P157 per total VASP was 29.2±3.4% before cilostazol intake at 0.5 h after PGE1 treatment, the ratio was increased at 3 h after cilostazol intake to 44.7±3.17% (p= 0.001) (Fig 2B). At 0.5 h after PGE1 treatment, VASP-P239 before cilostazol intake was 0.19±0.02 (arbitrary units), while at 3 h after cilostazol intake it was 0.82±0.22 (p< 0.001) (Fig 2C). Similarly, at 0.5 h after PGE1 treatment, VASP-P157/239 before cilostazol intake was 0.05±0.03 (arbitrary units), while at 3 h after cilostazol intake it was 0.93±0.19 (p<0.001) (Fig 2D). The effect of cilostazol intake on VASP phosphorylation under PGE1 treatment lasted for 12 h (Figs 2B–D). Although VASP-P157/239 under 8 nmol/L PGE1 for 0.5 h was barely detectable (0.05±0.01 arbitrary units) without cilostazol intake (Figs 2A,D), at 3 and 12 h after cilostazol intake it increased 18.6-fold (0.93±0.19, p=0.001) and 15.4-fold (0.77±0.24, p=0.016), respectively (Fig 2D). Therefore, the effects of cilostazol were most clearly detected in the evaluation of VASP phosphorylated at both Ser157 and Ser239, so the effect of cilostazol intake could be clearly detected by analyzing VASP phosphorylation under PGE1 treatment.

Cilostazol Intake Reduced Aggregability of PGE1-Treated Platelets

We next examined platelet aggregability under PGE1 treatment. Without cilostazol intake, the 5 μmol/L ADP-induced MAR without PGE1 treatment was 76.7±2.12% and at 1 h after 8 nmol/L PGE1 treatment was 52.4±5.56% (p<0.001) (Fig 4A). On the other hand, for platelets at 3 h after cilostazol intake, the 5 μmol/L ADP-induced MAR without PGE1 treatment was 76.9±1.13% and that at 1 h after 8 nmol/L PGE1 treatment was drastically decreased to 22.0±2.60% (p<0.001) (Fig 4A). Without PGE1 treatment, cilostazol intake did not decrease the MAR (76.7% vs 76.9%, p=0.932). Under PGE1 treatment, however, cilostazol intake significantly decreased the 5 μmol/L ADP-induced MAR (52.4% vs 22.3%, p<0.001) (Fig 4B). When the stimulus was 20 μmol/L ADP (data not shown), without cilostazol intake the MAR without PGE1 treatment was 81.7±2.3% and that at 1 h after 8 nmol/L PGE1 treatment was 69.8±3.59% (p= 0.004), whereas for platelets at 3 h after cilostazol intake, the MAR without PGE1 treatment was 82.2±1.61% and that at 1 h after 8 nmol/L PGE1 treatment was 48.7±5.58% (p<0.001). As with 5 μmol/L ADP, cilostazol intake significantly decreased the 20 μmol/L ADP-induced MAR under PGE1 treatment (69.8% vs 48.7%, p=0.001). Similar results were obtained with 1 μg/ml collagen as the stimulus, where without cilostazol intake the MAR without PGE1 treatment was 83.0±1.35% and that at 1 h after 8 nmol/L PGE1 treatment was 57.4±8.22% (p=0.009) (Fig 5A), whereas the MAR without PGE1 treatment was 82.4±1.16% and that at 1 h after 8 nmol/L PGE1 treatment was 13.7±2.41% (p<0.001) for platelets 3 h after cilostazol intake (Fig 5A). Under PGE1 treatment, cilostazol intake significantly decreased the MAR (57.4% vs 13.7%, p<0.001) (Fig 5B). Thus, we could clearly detect the effect of cilostazol intake by analyzing ADP- and collagen-induced platelet aggregation under PGE1 treatment.

Correlation of Cilostazol Levels in the Plasma With VASP Phosphorylation and Aggregation of Platelets

The plasma concentrations of cilostazol in all subjects were below the limit of detection (<20 ng/ml) at baseline, but were 732±89.9 ng/ml and 192±22.7 ng/ml at 3 h and 12 h, respectively, after 100 mg cilostazol intake (Fig 6A); these findings were similar to those reported previously[26]

Individual plots of cilostazol levels and VASP-P157 levels are shown in Fig 6B. Assessed by longitudinal analysis, considering change of cilostazol plasma concentration over time, VASP-P157 levels induced with 8 nmol/L PGE1 for 3 h strongly correlated with the plasma concentration of cilostazol (p=0.001) (Fig 6A). Because the values related to VASP-P239 and VASP-P157/239 were expressed as rela-