Tailored Adjunctive Cilostazol Therapy Based on CYP2C19 Genotyping in Patients With Acute Myocardial Infarction
—The CALDERA-GENE Study—

Koichi Kaikita, MD, PhD; Hiromi Yoshimura, MD, PhD; Masanobu Ishii, MD, PhD; Takashi Kudoh, MD, PhD; Yoshihiro Yamada, MD, PhD; Eiichiro Yamamoto, MD, PhD; Yasuhiro Izumiya, MD, PhD; Sunao Kojima, MD, PhD; Hideki Shimomura, MD, PhD; Ryusuke Tsunoda, MD, PhD; Kunihiiko Matsu, MD; Hisao Ogawa, MD, PhD; Kenichi Tsujita, MD, PhD for the CALDERA-GENE Investigators

**Background:** Patients with reduced-function CYP2C19 genotypes on dual antiplatelet therapy (DAPT) with aspirin and clopidogrel show higher clinical risk for acute myocardial infarction (AMI). We investigated the effect of CYP2C19 genotype-tailored adjunctive cilostazol therapy on treatment of AMI.

**Methods and Results:** The study group of 138 patients with suspected AMI were screened for CYP2C19 genotype immediately after percutaneous coronary intervention (PCI) using a SPARTAN RX point-of-care device. Carriers of the CYP2C19 reduced-function allele were randomized into DAPT (Carrier/DAPT) and DAPT plus 14-day cilostazol (Carrier/DAPT+Cilostazol) groups, while noncarriers were treated with DAPT (Noncarrier/DAPT). After exclusion of 10 patients, the remaining 128 patients were analyzed for P2Y12 reaction unit (PRU) using VerifyNow® P2Y12 system, and levels of biomarkers immediately after, and 1, 14, and 28 days after PCI. DAPT+Cilostazol reduced PRU levels in carriers (n=46) to those found in the Noncarrier/DAPT group (n=40), and significantly lower than those of the Carrier/DAPT group (n=42) at 14 days post-PCI. Discontinuation of cilostazol for 14 days was associated with a significant rise in PRU levels to those of the Carrier/DAPT group at 28 days post-PCI. Plasma B-type natriuretic peptide levels at 14 days post-PCI were lower in Carrier/DAPT+Cilostazol than in the other 2 groups, and the levels increased to those of the other groups at 28 days post-PCI after withdrawal of cilostazol.

**Conclusions:** Adjunctive cilostazol therapy tailored to CYP2C19 genotype seemed useful in AMI patients with the CYP2C19 reduced-function allele.

**Key Words:** Acute myocardial infarction; Antiplatelet therapy; Cilostazol; CYP2C19; Platelet aggregation

Dual antiplatelet therapy (DAPT) of aspirin and a P2Y12 inhibitor (clopidogrel, prasugrel, or ticagrelor) is essential for the regulation of activated platelets in disrupted coronary plaques in patients with acute myocardial infarction (AMI) and for reducing possible atherothrombotic complications in patients undergoing percutaneous coronary intervention (PCI). Observational studies have demonstrated that a high level of on-clopidogrel platelet aggregation is associated with adverse cardiovascular events after PCI. In this regard, patients with acute coronary syndrome undergoing PCI confirmed to be carriers of at least 1 reduced-function cytochrome P450 2C19 (CYP2C19) allele and treated with the DAPT of aspirin and clopidogrel have diminished platelet inhibition and a high rate of major adverse cardiovascular events.

CYP2C19 is one of the principal enzymes involved in the hepatic bioactivation of clopidogrel. Based on the clinical
outcomes of patients with AMI undergoing primary PCI, previous studies have advocated careful monitoring of on-clopidogrel platelet aggregation using various platelet function assays.\textsuperscript{12,13} We have also demonstrated the efficacy of monitoring of on-clopidogrel platelet aggregation by showing the cutoff levels of on-clopidogrel platelet aggregation that discriminated carriers of functional CYP2C19 from those with reduced-function allele among patients with stable coronary artery disease (CAD) treated with clopidogrel.\textsuperscript{14} We also reported that the platelet function tests, but not coagulation, inflammatory or cardiac biomarkers, seem to be useful for identifying carriers of CYP2C19 reduced-function gene variants and for monitoring the efficacy of DAPT in patients undergoing elective PCI.\textsuperscript{15} However, it is unclear whether CYP2C19 genotype-tailored antiplatelet therapy with adjunctive cilostazol is effective in inhibiting platelet aggregation in AMI patients with CYP2C19 reduced-function alleles. We conducted the present CALDERA-GENE (Controlled Anti-pLatelet meDical thErapy based on Rapid CYP2C19 GENE Evaluation in acute myocardial infarction; UMIN000008151) study, an open-label, prospective multicenter study that enrolled Japanese AMI patients who underwent primary PCI.

**Methods**

**Study Population and Study Protocol**

The CALDERA-GENE study is a prospective, multicenter, randomized and open-label study of 138 consecutive patients with suspected AMI (ST elevation and non-ST elevation MI) who underwent primary PCI between April 2013 and January 2016 at 3 Japanese cardiovascular institutions (Kumamoto University Hospital, Japanese Red Cross Kumamoto Hospital, and Fukuoka Tokushukai Medical Center). The patients fulfilled the following inclusion criteria: (1) admission within 12 h of symptom onset; (2) coronary angiography performed immediately after admission; and (3) primary PCI. AMI was based on the universal definition.\textsuperscript{16} None of the patients had thromboembolism, collagen disease, disseminated intravascular coagulation, septicemia, other inflammatory disease, or severe liver or renal dysfunction. Furthermore, none of the patients was on treatment with warfarin, steroids, thrombolytic agents, ticlopidine, sarpogrelate or cilostazol before PCI. We excluded patients who required mechanical cardiopulmonary support, such as intracorporeal balloon pumping (IABP) or percutaneous cardiopulmonary support (PCPS).

The risk factors for CAD were defined as current smoking (smoking within 1 year), diabetes mellitus (symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dL, fasting plasma glucose concentration ≥126 mg/dL, 2-h plasma glucose concentration ≥200 mg/dL during 75 g oral glucose tolerance test, or taking antidiabetic medications), hypertension (>140/90 mmHg or taking antihypertensive medications), dyslipidemia [high-density lipoprotein (HDL) cholesterol <40 mg/dL, low-density lipoprotein (LDL) cholesterol ≥140 mg/dL, or triglycerides ≥150 mg/dL or taking medications for dyslipidemia], and family history of MI.

Emergency coronary arteriography and primary PCI were performed on admission in AMI patients within 12 h of the onset of symptoms. AMI patients with implanted stents on admission were treated with both aspirin (loading dose of 200 mg/day followed by 100 mg/day) and clopidogrel (loading dose of 300 mg followed by 75 mg/day) just before PCI.

All patients were screened for CYP2C19 genotype immediately after PCI using the SPARTAN RX point-of-care device (Spartan Bioscience, Ottawa, ON, Canada).\textsuperscript{17} Patients who were carriers of CYP2C19 reduced-function alleles were randomized into 2 groups: those treated with DAPT (Carrier/DAPT group, aspirin+clopidogrel) or DAPT plus adjunctive 14-day cilostazol (200 mg/day) (Carrier/ DAPT+Cilostazol group); noncarriers were treated with DAPT (Noncarrier/DAPT group, aspirin+clopidogrel).

The study protocol was approved by the human ethics review committees of the 3 medical facilities at which this study was conducted, and a signed consent form was obtained from each subject. This study was financially supported by Daiichi Sankyo Co., Ltd.

**Platelet Function Tests**

These tests were conducted immediately after (approximately 2–3 h after oral administration of loading DAPT), and 1, 14 (end of treatment), and 28 days (14 days after end of treatment) after primary PCI. On-clopidogrel platelet aggregation was measured using the VerifyNow\textsuperscript{R} P2Y12 system (ULtra rapid platelet function assay; Accuretics Inc., San Diego, CA, USA). Briefly, for the VerifyNow\textsuperscript{R} assay, blood samples for each time point in the P2Y12 cartridge were withdrawn into 2 1.8-mL blood collection tubes each containing 3.2% sodium citrate and were analyzed independently. Fibrinogen-coated microparticles are used in the P2Y12 cartridge to bind to available platelet receptors.\textsuperscript{18} The results are reported in P2Y12 reaction units (PRU), which represent the amount of ADP-mediated aggregation specific to the platelet P2Y12 receptor. PRU are determined based on the rate and extent of platelet reactivity (by way of aggregation) in the ADP channel. Another separate channel contains fibrinogen-coated polystyrene beads and iso-thrombin receptor-activating peptide (iso-TRAP) as an agonist, and a baseline value (BASE) is obtained. The %inhibition is calculated as: \[
\frac{[\text{BASE−PRU}]\times 100}{\text{BASE}}
\]
which represents the difference between the pre- and post-treatment values.

**Measurement of Biomarkers of Blood Coagulation, Inflammation, and Myocardial Injury**

Venous blood samples were obtained immediately after, and 1, 14, and 28 days after primary PCI. All blood samples were immediately centrifuged at 1,700 g for 10 min at 4°C, and aliquots of samples were stored at −80°C until analysis.

Plasma von Willebrand factor (VWF) antigen levels were measured by the STA Liatest\textsuperscript{R} 1 VWF kit (Axis-Shield, Dundee, UK) according to the instructions supplied by the manufacturer. The serum levels of soluble P-selectin (sP-selectin) and soluble CD40 ligand (sCD40L) were measured by ELISA kits (sP-selectin: Takara Shuzo Co, Tokyo, sCD40L: R&D Systems, Oxford, UK) according to the manufacturers' instructions. Plasma cardiac troponin I (cTnI) was measured by high-sensitivity (hs)-TnI on Architecht i2000 (SR) (Abbott Diagnostics, Abbott Park, IL, USA), and serum cardiac troponin T (cTnT) was measured by hs-TnT on a cobas e602 analyzer (Roche Diagnostics, Mannheim, Germany). Plasma B-type natriuretic peptide (BNP) levels were measured using Architecht i2000 (SR) (Abbott Diagnostics).
Effects of Cilostazol on CYP2C19 Polymorphism

Determinations of CYP2C19 Genotype and Phenotype

Polymorphisms of CYP2C19 *2, *3, *17 were determined by the Spartan RX CYP2C19 point-of-care device. In brief, after rinsing the patient’s mouth with water, we opened all 3-sample collection kits (blue for CYP2C19 *2, white for CYP2C19 *3, and black for CYP2C19 *17) and took the buccal swabs and reagent tubes out of the kits. Each patient’s oral mucosa was scraped using the colored tip of the buccal swabs, which were placed into reagent tubes and analyzed with the Spartan RX Analyzer. The time from sample to result was approximately 60 min.

The CYP2C19 genotype was classified into the following phenotypes: ultra-rapid metabolizer (UM, including *17/*17, *1/*17), extensive metabolizer (EM, including *1/*1), intermediate metabolizer (IM, including *1/*2, *1/*3, *2/*17, *3/*17), and poor metabolizer (PM, including *2/*2, *2/*3, *3/*3).

Endpoints

The primary endpoints were: (1) the frequency of CYP2C19 *2, *3, and *17 polymorphism in AMI patients; and (2) DIFFERENCES in residual platelet aggregation, and biomarkers for blood coagulation, inflammation, and myocardial injury among the 3 treatment groups at the 4 preselected time points (immediately after, and 1, 14, and 28 days after primary PCI).

The secondary endpoints were the occurrence of cardiovascular events during the 1-month follow-up period after AMI. The composite of events included any of the following: cardiac death, fatal and non-fatal stroke, non-fatal MI, hospitalization for cardiovascular events, PCI or coronary artery bypass graft, hospitalization for heart failure, deep vein thrombosis, pulmonary thromboembolism, hospitalization for peripheral arterial disease, and hemorrhagic complications.

Statistical Analysis

Based on the results of the ACCEL-AM1-2C19 study,19 the PRU for the group with CYP2C19 reduced-function alleles treated with DAPT plus cilostazol at 2 weeks after AMI was assumed to be 23 ± 8, and the PRU for the group with CYP2C19 reduced-function alleles treated with DAPT was considered to be 29 ± 8 (from the results of the CALDERA-PCI study).12 The sample size of the 2 groups was determined by 2-tailed unpaired Student’s t-test with significance level set at 0.0166, which was calculated by Bonferroni correction, with a power level of 0.80, a mean difference in those groups of 57.4, and the common within-group standard deviation of 83.8, as mentioned before. The required sample size was 47 in each group. Previous study showed that approximately 60% of the East Asian population has CYP2C19 polymorphism. Finally, the total required sample size was 157 patients, including 63 noncarriers and 94 carriers of the CYP2C19 reduced-function alleles.

Data are expressed as mean ± standard deviation or median values (interquartile range: IQR). Categorical data are presented as frequencies and percentages. Differences between carriers and noncarriers were tested with the chi-square test (and Fisher exact test) for categorical variables. Differences in continuous variables were analyzed by the unpaired t-test and Mann-Whitney U test, or the 1-way analysis of variance (ANOVA) or Kruskal-Wallis test followed by multiple comparisons with the Bonferroni method, as appropriate. The statistical significance of changes in platelet function tests and biomarkers between carriers and noncarriers were evaluated by 2-way ANOVA with repeated measures followed by multiple comparisons with the Bonferroni method. P<0.05 denoted statistical significance. Statistical analyses were performed using The Statistical Package for Social Sciences version 23 (IBM Corporation, Armonk, NY, USA).

Results

Frequency of CYP2C19 Genotypes and Phenotypes, and Patient Characteristics

Figure 1 is a flow diagram of the patient recruitment
process. Among 138 consecutive patients with suspected AMI who were screened for CYP2C19 genotyping immediately after primary PCI with the SPARTAN RX point-of-care device, 93 carriers of CYP2C19 reduced-function allele were randomized into 2 groups. We excluded 10 patients for the following reasons: unstable angina pectoris (n=2; 1 in Noncarrier/DAPT, and 1 in Carrier/DAPT), incomplete sampling (n=7; 4 in Noncarrier/DAPT, 2 in Carrier/DAPT, and 1 in Carrier/DAPT+Cilostazol), and consent withdrawal (n=1 in Carrier/DAPT). The remaining 128 AMI patients (n=40 in Noncarrier/DAPT, n=42 in Carrier/DAPT, and n=46 in Carrier/DAPT+Cilostazol) were the subjects of the present study [115 men, 13 women; 88 in Carrier/DAPT, and 46 in Carrier/DAPT+Cilostazol].

Table 1. Baseline Clinical Characteristics of the Study Patients

| Characteristics                  | Noncarrier/DAPT (n=40) | Carrier/DAPT (n=42) | Carrier/DAPT+Cilostazol (n=46) | P value       |
|----------------------------------|------------------------|---------------------|--------------------------------|---------------|
| Age (years)                      | 62.6±12.4              | 62.8±10.3           | 64.5±12.6                      | >0.99 0.704   |
| Male, n (%)                      | 37 (92.5)              | 39 (92.9)           | 39 (84.8)                      | 0.591 0.364   |
| BMI (kg/m²)                      | 26.8±11.1              | 24.8±3.9            | 25.7±4.5                       | >0.99 0.471   |
| Hypertension, n (%)              | 29 (72.5)              | 27 (64.3)           | 29 (63.0)                      | >0.99 0.612   |
| Diabetes, n (%)                  | 14 (35.0)              | 14 (33.3)           | 15 (32.6)                      | >0.99 0.972   |
| Dyslipidemia, n (%)              | 17 (42.5)              | 19 (45.2)           | 28 (60.9)                      | 0.426 0.178   |
| Current smoker, n (%)            | 15 (37.5)              | 21 (50.0)           | 22 (47.8)                      | >0.99 0.478   |
| Hematocrit (%)                   | 42.9±5.2               | 42.0±8.3            | 42.9±4.4                       | >0.99 0.758   |
| Platelet count (10³/μL)          | 21.0±5.3               | 20.8±5.1            | 20.8±5.3                       | >0.99 0.988   |
| eGFR (ml/min/1.73 m²)            | 68.7±23.0              | 72.6±15.3           | 72.5±20.3                      | >0.99 0.602   |
| Total cholesterol (mg/dL)        | 195 [162–221]          | 189 [157–216]       | 209 [176–230]                  | 0.135 0.121   |
| LDL-C (mg/dL)                    | 114 [97–143]           | 116 [98–140]        | 122 [103–155]                  | 0.903 0.339   |
| HDL-C (mg/dL)                    | 47 [39–56]             | 40 [35–46]          | 42 [36–52]                     | 0.972 0.082   |
| Triglyceride (mg/dL)             | 102 [54–187]           | 110 [71–199]        | 111 [61–222]                   | >0.99 0.649   |
| FBG (mg/dL)                      | 165±68                 | 164±65              | 166±75                         | >0.99 0.982   |
| Hemoglobin A1c (%)               | 6.4±1.3                | 6.1±1.0             | 6.6±1.4                        | 0.304 0.259   |
| hs-CRP (mg/L)                    | 1.5 [0.5–3.4]          | 1.7 [0.7–2.8]       | 1.8 [1.0–3.9]                  | 0.750 0.343   |
| Peak CK (IU/L)                   | 2,220 [1,119–4,272]    | 2,164 [1,294–3,993] | 2,051 [1,025–3,300]            | >0.99 0.675   |
| Peak CK-MB isoenzyme (IU/L)      | 211 [101–336]          | 212 [108–340]       | 179 [102–385]                  | >0.99 0.898   |
| LVEF (%)                         | 53.1±9.2               | 49.6±8.2            | 51.5±6.8                       | 0.887 0.179   |
| PT-INR                           | 1.05±0.25              | 1.05±0.23           | 1.06±0.14                      | >0.99 0.977   |
| APTT (s)                         | 49.4±48.7              | 42.7±45.3           | 39.1±34.7                      | >0.99 0.545   |

Infarct-related artery

| Left anterior descending, n (%)  | 24 (60.0)              | 27 (64.3)           | 25 (54.3)                      | >0.99 0.635   |
| Left circumflex, n (%)           | 5 (12.5)               | 4 (9.5)             | 7 (15.2)                       | >0.99 0.722   |
| Right coronary, n (%)            | 11 (27.5)              | 11 (26.2)           | 14 (30.4)                      | >0.99 0.902   |
| Left main, n (%)                 | 0 (0.0)                | 0 (0.0)             | 1 (2.2)                        | >0.99 0.407   |
| Multivessel, n (%)               | 5 (12.5)               | 6 (14.3)            | 9 (19.6)                       | >0.99 0.639   |
| STEMI, n (%)                     | 36 (90.0)              | 40 (95.2)           | 40 (87.0)                      | 0.495 0.407   |
| Use of drug-eluting stent, n (%)  | 15 (37.5)              | 14 (33.3)           | 17 (37.0)                      | >0.99 0.911   |
| Onset-to-balloon time (min)      | 200 [117–268]          | 190 [143–321]       | 207 [145–299]                  | >0.99 0.588   |
| Door-to-balloon time (min)       | 69.0 [47.8–99.5]       | 68.5 [56.8–97.0]    | 59.0 [45.0–81.8]               | 0.276 0.260   |

Data are mean±SD or n (%). *Comparison of the 3 groups (Noncarrier/DAPT vs. Carrier/DAPT vs. Carrier/DAPT+Cilostazol). APTT, activated partial thromboplastin time; BMI, body mass index; CK, creatine kinase; DAPT, dual antiplatelet therapy of aspirin and clopidogrel; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; PT-INR, prothrombin time-international normalized ratio; STEMI, ST-segment elevation myocardial infarction.

respectively. The distribution of CYP2C19 phenotypes was 0 (0%), 40 (31%), 68 (53%), and 20 (16%) for UM (including *17/*17, *1/*17), EM (including *1/*1), IM (including *1/*2, *1/*3, *2/*17, *3/*17), and PM (including *2/*2, *2/*3, *3/*3), respectively. Thus, there were 88 cases (69%) of carriers of at least 1 CYP2C19 reduced-function allele [IM: 68 (53%), PM: 20 (16%)], and 40 cases (31%) of noncarriers (EM).

The clinical characteristics of the 3 treatment groups are listed in Table 1. There were no significant differences among the treatment groups in baseline characteristics known to be associated with platelet aggregation. Table 2 lists the medication history in each group before and after primary PCI. The proportions of patients on nitrates, statins, β-blockers, calcium-channel blockers, antidiabetic agents, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and proton pump inhibitors, were identical among the 3 treatment groups before and
Effects of Cilostazol on CYP2C19 Polymorphism

%inhibition levels increased significantly in the Carrier/DAPT+Cilostazol compared with the Carrier/DAPT groups (PRU: 142 ± 58 vs. 180 ± 49, P=0.010, %inhibition: 47.1 ± 21.5 vs. 28.5 ± 16.3, P<0.001), and were not different from those of the Noncarrier/DAPT group (PRU: 142 ± 58 vs. 146 ± 71, P=1.00, %inhibition: 47.1 ± 21.5 vs. 44.5 ± 25.2, P=1.00) 14 days after primary PCI. After discontinuation of cilostazol at day 14, the PRU levels increased and the %inhibition levels increased significantly in the Carrier/DAPT+Cilostazol compared with the Carrier/DAPT groups (PRU: 142±58 vs. 180±49, P=0.010, %inhibition: 47.1±21.5 vs. 28.5±16.3, P<0.001), and were not different from those of the Noncarrier/DAPT group (PRU: 142±58 vs. 146±71, P=1.00, %inhibition: 47.1±21.5 vs. 44.5±25.2, P=1.00) 14 days after primary PCI. After discontinuation of cilostazol at day 14, the PRU levels increased and the

### Table 2. Medication History in the 3 Treatment Groups Before and After Primary PCI

| Medications before PCI | Noncarrier/DAPT (n=40) | Carrier/DAPT (n=42) | Carrier/DAPT+Cilostazol (n=46) | P value* | P value** |
|------------------------|------------------------|---------------------|---------------------------------|----------|-----------|
| Nitrates, n (%)        | 1 (2.5)                | 3 (7.1)             | 2 (4.3)                         | >0.99    | 0.604     |
| Statins, n (%)         | 8 (20.0)               | 6 (14.6)            | 5 (10.9)                        | >0.99    | 0.495     |
| β-blockers, n (%)      | 5 (12.5)               | 4 (9.5)             | 0 (0.0)                         | 0.144    | 0.058     |
| CCB, n (%)             | 16 (40.0)              | 14 (33.3)           | 9 (19.6)                        | 0.426    | 0.108     |
| Antidiabetic agents, n (%) | 10 (25.0)         | 9 (21.4)            | 6 (13.0)                        | 0.888    | 0.352     |
| ACEI, n (%)            | 1 (2.5)                | 1 (2.4)             | 2 (4.3)                         | >0.99    | 0.837     |
| ARB, n (%)             | 16 (40.0)              | 10 (23.8)           | 9 (19.6)                        | >0.99    | 0.087     |
| PPI, n (%)             | 3 (7.5)                | 2 (4.8)             | 3 (6.5)                         | >0.99    | 0.873     |

| Medications after PCI | Noncarrier/DAPT (n=40) | Carrier/DAPT (n=42) | Carrier/DAPT+Cilostazol (n=46) | P value* | P value** |
|-----------------------|------------------------|---------------------|---------------------------------|----------|-----------|
| Nitrates, n (%)       | 1 (2.6)                | 2 (4.8)             | 1 (2.2)                         | >0.99    | 0.761     |
| Statins, n (%)        | 38 (95.0)              | 37 (88.1)           | 39 (84.8)                       | >0.99    | 0.308     |
| β-blockers, n (%)     | 29 (72.5)              | 23 (54.8)           | 31 (67.4)                       | 0.672    | 0.220     |
| CCB, n (%)            | 6 (15.0)               | 6 (14.3)            | 7 (15.2)                        | >0.99    | 0.982     |
| Antidiabetic agents, n (%) | 8 (20.0)            | 10 (23.8)           | 7 (15.2)                        | 0.924    | 0.595     |
| ACEI, n (%)           | 24 (61.5)              | 25 (59.5)           | 28 (62.2)                       | >0.99    | 0.985     |
| ARB, n (%)            | 10 (25.6)              | 9 (21.4)            | 9 (20.0)                        | >0.99    | 0.816     |
| PPI, n (%)            | 34 (87.2)              | 38 (90.5)           | 44 (95.7)                       | 0.888    | 0.373     |

Data are n (%). *Comparison of the 3 groups (Noncarrier/DAPT vs. Carrier/DAPT vs. Carrier/DAPT+Cilostazol). ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor antagonists; CCB, calcium-channel blockers; DAPT, dual antiplatelet therapy of aspirin and clopidogrel; PCI, percutaneous coronary intervention; PPI, proton pump inhibitors.

**Figure 2.** Serial changes in platelet inhibition measured by VerifyNow® P2Y12 system [(A) PRU and (B) %inhibition] immediately after, and 1, 14, and 28 days after primary percutaneous coronary intervention. PRU, P2Y12 reaction unit; DAPT, dual antiplatelet therapy of aspirin and clopidogrel; On-Cilostazol, while on treatment with cilostazol in the Carrier/DAPT+Cilostazol group; Off, cilostazol withdrawal in the Carrier/DAPT+Cilostazol group. *P<0.05, Bonferroni’s multiple comparison test, Carrier/DAPT vs. Noncarrier/DAPT and Carrier/DAPT+Cilostazol. †P<0.01, Bonferroni’s multiple comparison test, Noncarrier/DAPT vs. Carrier/DAPT and Carrier/DAPT+Cilostazol. Data are mean±SD.
%inhibition levels decreased significantly in the Carrier/DAPT+Cilostazol compared with the Noncarrier/DAPT groups (PRU: 197±56 vs. 143±65, P<0.001, and %inhibition: 28.8±16.8 vs. 45.4±25.6, P=0.001), and were not different from those of the Carrier/DAPT group at 28 days post-PCI (PRU: 197±56 vs. 192±54, P=1.00, and %inhibition: 28.8±16.8 vs. 26.7±17.9, P=1.00).

Serial Changes in Biomarkers of Blood Coagulation, Inflammation, and Myocardial Injury

Figure 3 demonstrates the serial changes in blood levels of various biomarkers of coagulation activation, inflammation, and myocardial injury in the 3 study groups. Plasma VWF and hs-TnI, and serum sP-selectin, sCD40L, and hs-TnT levels were almost identical throughout the study among the 3 groups, except for higher VWF levels in the Noncarrier/DAPT compared with the Carrier/DAPT+Cilostazol group at day 1 after primary PCI. Interestingly, plasma BNP levels decreased significantly at 14 days post-PCI in the Carrier/DAPT+Cilostazol group (40.6 [20.1–67.7]) compared with the other 2 groups (vs. 73.3 [23.1–169.9], P=0.019, compared with Noncarrier/DAPT group; vs. 95.3 [38.7–165.4], P=0.001, compared with Carrier/DAPT group). After discontinuation of cilostazol at day 14, the plasma BNP level in the Carrier/DAPT+Cilostazol group (63.8 [34.4–125.1]) was as high as those of the Noncarrier/DAPT (61.6 [24.3–111.3]) and Carrier/DAPT groups (63.3 [31.8–107.2]) at 28 days post-PCI (P=0.81).

Clinical Outcomes

During the 1-month follow-up period, composite cardiovascular events occurred in 2 (5.0%) patients of the Noncarrier/DAPT group (non-fatal MI: n=1; heart failure: n=1), 2 (4.8%) patients of the Carrier/DAPT group (non-fatal MI: n=2), and in 1 (2.2%) patient of the Carrier/DAPT+Cilostazol group (unstable angina). During the 1-month follow-up period, no major bleeding complications occurred in the 3 treatment groups. A minor bleeding complication from a hemorrhoid occurred in 1 (2.4%)
Effects of Cilostazol on CYP2C19 Polymorphism

1523

patient of the Carrier/DAPT group. Also, headache occurred in 2 (4.4%) patients and palpitations in 2 (4.4%) patients of the Carrier/DAPT+Cilostazol group.

**Discussion**

The present study demonstrated that CYP2C19 genotype-tailored adjunctive cilostazol therapy effectively suppressed on-clopidogrel platelet aggregation and plasma BNP levels in AMI patients with CYP2C19 reduced-function genotypes. Recent clinical studies demonstrated that prasugrel and ticagrelor achieved greater and more consistent platelet inhibition than standard doses of clopidogrel.20,21 Although adjunctive cilostazol might be an alternative regimen to overcome the risk of high platelet aggregability in carriers of the CYP2C19 variants, it has been reported that this regimen significantly enhances platelet inhibition and reduces the rate of high on-clopidogrel platelet reactivity, especially in AMI patients with CYP2C19 loss-of-function variants.19 To the best of our knowledge, this is the first report demonstrating that adjunctive cilostazol therapy tailored to CYP2C19 genotype appeared to be clinically useful in AMI patients with CYP2C19 reduced-function alleles.

We evaluated the point-of-care method of genotyping CYP2C19 polymorphisms by the Spartan RX CYP2C19 test, which became available recently for CYP2C19 *2, *3, and *17 alleles. This test identified 88 cases (69%) as carriers of at least 1 CYP2C19 reduced-function allele [IM: 68 (53%), PM: 20 (16%),] and 40 cases (31%) as noncarriers (EM). Interestingly, none of the patients had the UM phenotype. The frequency of each CYP2C19 genotype group differs among the races.22 Asians carry more loss-offunction alleles (30–35% in *2; 5–10% in *3) and less gain-of-function alleles (2–4% in *17) than Africans (17–20% in *2; <1% in *3; 18% in *17) and Caucasians (13–18% in *2; <1% in *3; 18–20% in *17). In the present study, the frequency of the carriers of at least 1 CYP2C19 reduced-function allele was almost similar to that reported earlier by our group in different population samples.14,15 We could not find CYP2C19 gain-of-function alleles (*17) in the present study, so clinical studies that include large numbers of subjects are needed to determine the true frequency of CYP2C19 gain-of-function alleles (*17) in Japanese AMI patients.

In the present study, the addition of cilostazol to DAPT significantly reduced PRU levels at day 14 after PCI in carrier patients compared with those observed in the Non-carrier/DAPT group. Interestingly, after discontinuation of cilostazol on day 14, PRU levels increased significantly at day 28 post-PCI to the same levels found in carriers treated with DAPT. Cilostazol inhibits platelet aggregation through selective inhibition of phosphodiesterase type 3 (PDE3) and results in increased levels of cyclic adenosine monophosphate (cAMP) in platelets.23 Recent studies showed that the addition of cilostazol to DAPT (i.e., triple antiplatelet therapy) resulted in greater ADP-induced platelet inhibition compared with DAPT.24,25 The present finding suggested that adjunctive cilostazol therapy could be an alternative regimen for achieving enhanced platelet inhibition in carriers of CYP2C19 reduced-function alleles.

Recent studies showed that carriers of at least 1 CYP2C19 reduced-function allele had significantly lower levels of active metabolites of clopidogrel, reduced platelet inhibition, and higher rates of major adverse cardiovascular events than noncarriers among acute coronary syndrome patients treated with clopidogrel for PCI.11,20 On the other hand, several studies have suggested that stronger engagement of platelet ADP P2Y12 receptor by ADP antagonists can increase the risk of bleeding,13 suggesting that the clinical efficacy of aggressive platelet inhibition by potent antiplatelet regimens can translate into reduced safety. In the present study, the frequency of composite cardiovascular events was low (3.9% in total), and no major bleeding complications occurred in the 3 treatment groups during the 28-day follow-up period. The reasons for no major bleeding complication in the Carrier/DAPT+Cilostazol group could be the short-term (2 weeks) cilostazol treatment and the enrollment of the patients with relatively mild AMI who did not need mechanical cardiopulmonary support such as IABP or PCPS. Clinical studies that examine the risk of bleeding during long-term follow-up are needed to determine the overall safety of adjunctive cilostazol therapy with DAPT in Japanese AMI patients.

Several biomarkers of platelet and coagulation activation, inflammation, and myocardial injury are thought to play important roles in the pathogenesis of atherothrombotic complications, such as acute or subacute stent thrombosis in AMI patients undergoing PCI. In this regard, a high clopidogrel maintenance dose was associated with stronger platelet inhibition, improved endothelial function, and reduced inflammation, compared with the currently recommended 75-mg/day regimen,26 and the latter clopidogrel regimen differentially affects platelet aggregation and platelet-induced inflammation in ACS patients within the first month of treatment.27 On the other hand, it has been shown that cilostazol also improves endothelial function by increasing the level of cAMP.28 Based on these observations, we hypothesized that adjunctive cilostazol therapy can alter the levels of various blood biomarkers of platelet and coagulation activation, inflammation, and myocardial injury in carriers of at least 1 CYP2C19 reduced-function allele with AMI on treatment with DAPT. In the present study, although plasma VWF and hs-TnI, and serum sP-selectin, sCD40L, and hs-TnT levels were almost identical in the 3 groups throughout the study, plasma BNP levels decreased significantly at 14 days post-PCI in carriers treated with DAPT plus cilostazol compared with the other 2 groups. To further confirm the effects of cilostazol, its discontinuation at day 14 resulted in a jump in plasma BNP within 2 weeks to levels equivalent to those encountered in the other 2 groups at 28 days post-PCI.

Cilostazol is a widely used selective and reversible inhibitor of PDE3, which is highly expressed in myocardial and vascular smooth muscle cells (VSMCs) and platelets. Cilostazol administered at clinically relevant concentrations also inhibits adenosine reuptake into erythrocytes, endothelial cells, muscle cells, and platelets, thereby increasing interstitial and circulatory adenosine levels.31 Inhibitors of PDE3 are used clinically to increase cardiac contractility by raising the intracellular cAMP content in cardiac myocytes, and to reduce vascular resistance by increasing intracellular cGMP content in VSMCs.32 In the present study, plasma BNP levels decreased significantly at 14 days post-PCI in the Carrier/DAPT+Cilostazol group compared with the other 2 groups. This finding suggested that cilostazol might improve plasma BNP levels by increasing the contractility of viable myocardium through raising the intracellular cAMP content and by reducing the vascular resistance. On the other hand, cilostazol did not affect the
plasma hs-TnI and serum hs-TnT levels in AMI patients after primary PCI. The corollary of this finding is that the increased myocardial cAMP content by cilostazol therapy might not contribute to the protection of myocardial injury after AMI. Also, in the treatment of patients with heart failure, PDE3 inhibitors are effective in the acute setting but increase the rate of sudden cardiac death with long-term administration, possibly reflecting pro-apoptotic and pro-hypertrophic consequences of increased cAMP-mediated signaling in cardiac myocytes. These mechanisms may explain the observed improvement in plasma BNP levels in the present patients on short-term (2 weeks) adjunctive cilostazol therapy with DAPT.

Study Limitations

First, because we enrolled patients with relatively mild AMI who did not need mechanical cardiopulmonary support, such as IABP or PCPS, the frequency of composite cardiovascular events was low in the 3 treatment groups. Large clinical studies with long-term follow-up are needed to determine the efficacy and safety of adjunct cilostazol therapy with DAPT in Japanese AMI patients. Second, the present study was conducted in relatively young and male AMI patients. Therefore, the observed effects of adjunct cilostazol therapy need to be confirmed in female AMI patients with CYP2C19 reduced-function alleles. Third, we could not include treatment arms of prasugrel and ticagrelor because those drugs were not available to use clinically in Japan when we started the patient enrollment for the present study.

Conclusions

In summary, our CALDERA-GENE study demonstrated that CYP2C19 genotype-based adjunctive cilostazol therapy effectively suppressed on-clopidogrel platelet aggregation and reduced plasma BNP levels in AMI patients with CYP2C19 reduced-function genotypes. These findings suggested that cilostazol is effective in Japanese AMI patients with CYP2C19 reduced-function alleles.

Acknowledgments

We are grateful to Satomi Iwashita, Megumi Nagahiro and Saeko Tokunaga from the Department of Cardiology, Kumamoto University, for their skillful technical assistance.

Disclosures

Dr. Kaikita has received several research grants from Bayer Yakuhin, Ltd., Daiichi Sankyo Co., Ltd., Novartis Pharma K.K., and SBI Pharma K.K. Dr. Tsuji has received honoraria from Amgen Ltd., Daiichi Sankyo Co., Ltd., Novartis Pharma K.K., and SBI Pharma K.K. Dr. Shirato has received several research grants from Bayer Yakuhin, Ltd., and also grants from AstraZeneca K.K., Astellas Pharma Inc., and MSD K.K., and also from grants from Daiichi Sankyo Co., Ltd., MSD K.K., Novo Nordisk A/S, and SBI Pharma K.K. Dr. Yokota has received several research grants from Daiichi Sankyo Co., Ltd., Novartis Pharma K.K., and also from grants from AstraZeneca K.K., Eisai Co., Ltd., Kowa Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma, MSD K.K., Pfizer Japan Inc., Sanofi K.K., and Takeda Pharmaceutical Co., Ltd. All other authors declare no conflicts of interest.

Authorship Contributions

K.K., and H.Y. conceived and designed the research; M.I., T.K., Y.Y., E.Y., Y.I., S.K., H.S., and R.T. performed the experiments; M.I. and K.M. analyzed the data; K.K. drafted, edited and revised manuscript; H.O. and K.T. approved the final version of manuscript.

Funding

This study was supported in part by research grants from Daiichi Sankyo Co., Ltd., Tokyo, Japan.

References

1. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 2007; 357: 2001 – 2015.
2. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 2009; 360: 1045 – 1057.
3. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: The PCI-CURE study. Lancet 2001; 358: 527 – 533.
4. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK, et al. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med 2001; 345: 494 – 502.
5. Marcucci R, Gori AM, Panucci R, Giusti B, Valente S, Giglioli C, et al. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay. Circulation 2009; 119: 237 – 242.
6. Sabatine MS, Cannon CP, Gibson CM, López-Sendón JL, Montalescot G, Theroux P, et al. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. N Engl J Med 2005; 352: 1179 – 1189.
7. Matejczyk S, Shenkman B, Guetta V, Shechter M, Beinart R, Goldenberg I, et al. Cilostazol reduces the immediate and short-term inhibition of platelet aggregation after loading with clopidogrel on early clinical outcome of elective coronary stent placement. J Am Coll Cardiol 2006; 48: 1742 – 1750.
8. Hohenhofer W, Trenk D, Bestehorn HP, Fischer B, Valina CM, Ferenc M, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. J Am Coll Cardiol 2006; 45: 1827 – 1832.
9. Trenk D, Hohenhofer W, Fromm MF, Chialda LE, Pahl A, Valina CM, et al. Cytochrome P450 2C19 617G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. J Am Coll Cardiol 2008; 51: 1925 – 1934.
10. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome P450 2C19 polymorphism and response to clopidogrel. N Engl J Med 2009; 360: 354 – 362.
11. Gurbel PA, Bleden KP, Guyer K, Cho PW, Zaman KA, Kreutz RP, et al. Platelet reactivity in patients and recurrent events post-stenting: Results of the PREPARE POST-STEMTING Study. J Am Coll Cardiol 2005; 46: 1820 – 1826.
12. Breit NJ, van Werkum JW, Bouman HJ, Kelder JC, Ruven HJ, Bal ET, et al. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. JAMA 2010; 303: 754 – 762.
13. Ono T, Kaikita K, Hokimoto S, Iwashita S, Yamamoto K, Miyazaki Y, et al. Determination of cut-off levels for on-clopidogrel platelet aggregation based on functional CYP2C19 gene variants in patients undergoing elective percutaneous coronary intervention. Thromb Res 2011; 128: e130 – e136.
14. Kaikita K, Ono T, Iwashita S, Nakayama N, Sato K, Horio E, et al. Impact of CYP2C19 polymorphism on platelet function tests and coagulation and inflammatory biomarkers in patients undergoing percutaneous coronary intervention. J Atheroscler Thromb 2014; 21: 64 – 76.
15. Thaygesen K, Alpert JS, Aaffe AS, Simoons ML, Chatrnan BR, White HD, et al. Third universal definition of myocardial infarction. Circulation 2012; 126: 2030 – 2035.
16. Choi JL, Kim BR, Woo KS, Kim KH, Kim JM, Kim MH, et al. The diagnostic utility of the point-of-care CY2CP2C19 genotyping assay in patients with acute coronary syndrome using clopidogrel. Comparison with platelet function test and SNP genotyping. Ann Clin Lab Sci 2016; 46: 489 – 494.
18. Malinin A, Pokov A, Spergling M, Defranco A, Schwartz K, Schwartz D, et al. Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: The VERITY Thrombosis risk ASeessment (VERITAS) study. *Thromb Res* 2011; 119: 277–284.

19. Jeong YH, Hwang JY, Kim IS, Park Y, Hwang SJ, Lee SW, et al. Adding cilostazol to dual antiplatelet therapy achieves greater platelet inhibition than high maintenance dose clopidogrel in patients with acute myocardial infarction: Results of the adjunctive cilostazol versus high maintenance dose clopidogrel in patients with AMI (ACCEL-AMI) study. *Circ Cardiovasc Interv* 2010; 3: 17–26.

20. Saito S, Ishiki T, Kimura T, Ogawa H, Yokoi H, Nanto S, et al. Efficacy and safety of adjusted-dose prasugrel compared with clopidogrel in Japanese patients with acute coronary syndrome: The PRASFIT-ACS study. *Circ J* 2014; 78: 1684–1692.

21. Garbel PA, Bilden KP, Butler K, Tantry US, Gesheff T, Wei C, et al. Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: The ONSET/OFFSET study. *Circulation* 2009; 120: 2577–2585.

22. Terpening C. Clopidogrel: A pharmacogenomic perspective on its use in coronary artery disease. *Clin Med Insights Cardiol* 2010; 4: 117–128.

23. Goto S. Cilostazol: Potential mechanism of action for antithrombotic effects accompanied by a low rate of bleeding. *Atheroscler Suppl* 2005; 6: 3–11.

24. Lee BK, Lee SW, Park SW, Lee SW, Park DW, Kim YH, et al. Effects of triple antiplatelet therapy with aspirin, clopidogrel and cilostazol on platelet aggregation and P-selectin expression in patients undergoing coronary artery stent implantation. *Am J Cardiol* 2007; 100: 610–614.

25. Angiolillo DJ, Capranzano P, Goto S, Aslam M, Desai B, Charlton RK, et al. A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: Results of the OPTIMUS-2 study. *Eur Heart J* 2008; 29: 2202–2211.

26. Jeong YH, Lee SW, Choi BR, Kim IS, Seo MK, Kwak CH, et al. Randomized comparison of adjunctive cilostazol versus high maintenance dose clopidogrel in patients with high post-treatment platelet reactivity: Results of the ACCEL-RESISTANCE (Adjunctive Cilostazol Versus High Maintenance Dose Clopidogrel in Patients With Clopidogrel Resistance) randomized study. *J Am Coll Cardiol* 2009; 53: 1101–1109.

27. Kim IS, Jeong YH, Park Y, Park KS, Yun SE, Park JR, et al. Platelet inhibition by adjunctive cilostazol versus high maintenance-dose clopidogrel in patients with acute myocardial infarction according to cytochrome P450 2C19 genotype. *JACC Cardiovasc Interv* 2011; 4: 381–391.

28. Mega JL, Simon T, Collet JP, Anderson JL, Antman EM, Bliden K, et al. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: A meta-analysis. *JAMA* 2010; 304: 1821–1830.

29. Patti G, Grieco D, Dicuonzo G, Pasceri V, Nusca A, Di Sciascio G. High versus standard clopidogrel maintenance dose after percutaneous coronary intervention and effects on platelet inhibition, endothelial function, and inflammation results of the ARMYDA-150 mg (antiplatelet therapy for reduction of myocardial damage during angioplasty) randomized study. *J Am Coll Cardiol* 2011; 57: 771–778.

30. Kalantzi KI, Dimitriou AA, Milionis HJ, Goudevenos IA, Tselepis AD. Clopidogrel differentially affects platelet-mediated thrombosis and inflammatory response in patients with acute coronary syndromes. *J Thromb Haemost* 2011; 9: 875–878.

31. Ota H, Eto M, Ako J, Ogawa S, Iijima K, Akishita M, et al. Sirolimus and everolimus induce endothelial cellular senescence via sirtuin 1 down-regulation: Therapeutic implication of cilostazol after drug-eluting stent implantation. *J Am Coll Cardiol* 2009; 53: 2298–2305.

32. Liu Y, Shakur Y, Kambayashi J. Phosphodiesterases as targets for intermittent claudication. *Handb Exp Pharmacol* 2011; 204: 211–236.

33. Movsesian M. Novel approaches to targeting PDE3 in cardiovascular disease. *Pharmacol Ther* 2016; 165: 74–81.