Clopidogrel Resistance in Japanese Patients Scheduled for Percutaneous Coronary Intervention

Kozo Hoshino, MD; Hisanori Horiuichi, MD; Tomohisa Tada, MD; Junichi Tazaki, MD; Eiichiro Nishi, MD; Mitsunori Kawato, MD; Tomoyuki Ikeda, MD; Hiromi Yamamoto, MD; Masaharu Akao, MD; Yutaka Furukawa, MD; Satoshi Shizuta, MD; Masanao Toma, MD; Toshihiro Tamura, MD; Naritatsu Saito, MD; Takahiro Doi, MD; Neiko Ozasa, MD; Toshikazu Jinnai, MD; Kanako Takahashi, MT; Haruyo Watanabe, MT; Yuka Yoshikawa, MT; Naoko Nishimoto, MT; Chiho Ouchi, MT; Takeshi Morimoto, MD*; Toru Kita, MD; Takeshi Kimura, MD

Background  Dual antiplatelet therapy with acetylsalicylic acid (ASA) and a P2Y12 ADP-receptor blocker is standard for prevention of coronary stent thrombosis. Clopidogrel, a 2nd-generation P2Y12 blocker, has recently become available in Japan and this study aimed to evaluate its antiplatelet effects in Japanese patients.

Methods and Results  Thirty Japanese patients scheduled for elective coronary stent implantation were enrolled. Under low-dose ASA therapy, 300 mg clopidogrel was loaded on the 1st day and a daily 75-mg dose was administered on the following days. Assessed by optical aggregometer, rapid inhibition occurred at 4 h, when the inhibition of platelet aggregation rate (IPA) was 16.4±12.8% using 5 μmol/L ADP as the stimulus. The antiplatelet efficacy of clopidogrel was reasonably constant in each patient throughout the study period, although there was a broad inter-individual variation. At 48 h after clopidogrel loading, the ratios of responders (IPA ≥30%), hypo-responders (10%≤IPA<30%), and non-responders (IPA <10%) were 36%, 50%, and 14%, respectively.

Conclusions  The antiplatelet effectiveness of clopidogrel appeared individual-specific with wide inter-individual variation. The rate of clopidogrel non-responders was 14% among the examined Japanese patients.  (Circ J 2009; 73: 336–342)

Key Words:  Adenosine diphosphate; Antiplatelet drug; Clopidogrel; Coronary stent; Thienopiridine

Percutaneous coronary intervention (PCI) with coronary stent implantation is performed worldwide for ischemic heart disease. In Japan, 153,501 patients underwent this therapy in 2006, as described in the surveillance report from the Japan Circulation Society. One of the most serious problems is acute and late thrombosis at the site of stenting and much effort had been made to avoid this critical complication. The current standard dual antiplatelet therapy with acetylsalicylic acid (ASA) and thienopyridine ADP-receptor blocker has proven to be a powerful preventive solution.

Two thienopyridine antiplatelet agents are currently available: ticlopidine and clopidogrel. Although ticlopidine, a 1st-generation thienopyridine, has contributed much to the prevention of stent thrombosis, it frequently causes adverse side-effects such as agranulocytosis, thrombotic thrombocytopenic purpura and liver injury. Clopidogrel, a 2nd-generation P2Y12 blocker, has a better safety profile with a lower incidence of hematologic and liver complications, and has now largely replaced ticlopidine in clinical practice.

One important problem with clopidogrel is the wide inter-individual variation in its antiplatelet effect. It has been demonstrated that clopidogrel does not exert an antiplatelet effect in a certain proportion of patients in Western populations, known as clopidogrel resistance. Importantly, several studies have revealed that cardiovascular risk is elevated in patients with clopidogrel resistance.

On the other hand, there are well-established differences in the atherothrombotic and hemorrhagic risks in the Japanese compared with Western populations, so results from clinical trials in the West using novel antithrombotic agents cannot be applied directly to Japanese patients. Furthermore, the standard dose of ticlopidine for the Japanese (200 mg/day) is much lower than that for Western people (500 mg/day), but the same daily maintenance dose (75 mg) of clopidogrel is used in both populations. Therefore, some Japanese physicians are concerned about the strength of the effect of clopidogrel in Japanese patients and because of those concerns, we designed the present study to evaluate the antiplatelet effects of clopidogrel under low-dose ASA therapy in 30 Japanese patients scheduled for PCI.

Methods

Study Protocol  This study was approved by the Ethics Committee of Kyoto University Hospital, and written informed consent was given by all enrolled patients, who were undergoing elective...
coronary stent implantation. The initial diagnosis of ischemic heart disease was based on symptoms, a non-invasive examination such as stress electrocardiogram, and/or coronary computed tomography angiography. Further entry criteria were: (1) ASA (81–100 mg/daily) for at least 7 days prior to the initial cardiac catheterization and (2) platelet count of 100–350 × 10^9/L and hemoglobin ≥10 g/dl. Exclusion criteria were: (1) recent bleeding diathesis; (2) hematologic or malignant disorder; (3) oral anticoagulation with coumarin derivatives; (4) glycoprotein IIb/IIIa inhibitor or fibrinolitics administered during either the PCI or the preceding 14 days; and (5) antiplatelet therapy with thienopyridines, cilostazol or dipyridimole within the preceding 28 days.

A loading dose of 300 mg clopidogrel was administered on the 1st day, approximately 24 h before PCI. A daily maintenance dose (75 mg) was administered the morning before the procedure and continued thereafter. ASA was administered at a daily dose of 81–100 mg. Blood samples were collected at enrolment, and at 4 (3–5) h, 24 (22–26) h and 48 (46–50) h after the loading dose (Table 1). The 24-h sampling was performed before the daily 75 mg clopidogrel intake, whereas the 48-h sampling was afterward. All 30 enrolled patients were to be evaluated until 48 h after the loading dose; 9 patients did not undergo PCI because of unexpected mild stenosis and re-evaluation of the PCI indication on the following day and therefore, those patients discontinued clopidogrel intake. PCI was carried out in the remaining 21 patients and their blood samples were analyzed on days 14 (12–16) and 28 (26–30).

### Analysis of Platelet Aggregation

Blood samples were collected using a 21G needle, with tourniquet, into a glass tube containing a final solution of 0.313% sodium citrate. Platelet-rich plasma (PRP) was prepared by centrifugation at 150 g at 30°C for 15 min and the PRPs were stimulated by 0.313% sodium citrate. Platelet-rich plasma (PRP) was collected at enrolment, and at 4 (3–5) h, 24 (22–26) h and 48 (46–50) h after the loading dose (Table 1). The 24-h sampling was performed before the daily 75 mg clopidogrel intake, whereas the 48-h sampling was afterward. All 30 enrolled patients were to be evaluated until 48 h after the loading dose; 9 patients did not undergo PCI because of unexpectedly mild stenosis and re-evaluation of the PCI indication on the following day and therefore, those patients discontinued clopidogrel intake. PCI was carried out in the remaining 21 patients and their blood samples were analyzed on days 14 (12–16) and 28 (26–30).

#### Table 1 Study Protocol

<table>
<thead>
<tr>
<th>Day</th>
<th>Platelet function analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Clopidogrel 300 mg</td>
</tr>
<tr>
<td>Day 1</td>
<td>Clopidogrel 75 mg</td>
</tr>
<tr>
<td>Day 28</td>
<td></td>
</tr>
</tbody>
</table>

#### Table 2 Baseline Characteristics

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Total (n=30)</th>
<th>PCI (n=21)</th>
<th>Non-PCI (n=9)</th>
<th>P (PCI vs non-PCI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>70±7</td>
<td>71±9</td>
<td>68±5</td>
<td>0.11</td>
</tr>
<tr>
<td>Males</td>
<td>22 (73%)</td>
<td>17 (81%)</td>
<td>5 (56%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Platelets (×10^4/μl)</td>
<td>21.1±5.5</td>
<td>20.4±5.2</td>
<td>22.2±5.8</td>
<td>0.43</td>
</tr>
<tr>
<td>Current smoker</td>
<td>10 (33%)</td>
<td>11 (52%)</td>
<td>0 (0%)</td>
<td>0.0013</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>22 (73%)</td>
<td>18 (86%)</td>
<td>5 (56%)</td>
<td>0.083</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (23%)</td>
<td>6 (29%)</td>
<td>1 (11%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Hypertension</td>
<td>18 (60%)</td>
<td>15 (71%)</td>
<td>4 (44%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>1 (3%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Prior PCI</td>
<td>4 (13%)</td>
<td>3 (14%)</td>
<td>1 (11%)</td>
<td>0.81</td>
</tr>
<tr>
<td>Prior cerebrovascular event</td>
<td>1 (3%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>2 (7%)</td>
<td>2 (10%)</td>
<td>0 (0%)</td>
<td>0.22</td>
</tr>
<tr>
<td>β-blocker</td>
<td>4 (13%)</td>
<td>3 (14%)</td>
<td>1 (11%)</td>
<td>0.81</td>
</tr>
<tr>
<td>Nitrates</td>
<td>7 (23%)</td>
<td>5 (24%)</td>
<td>2 (22%)</td>
<td>0.92</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>13 (43%)</td>
<td>8 (38%)</td>
<td>5 (56%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Statin</td>
<td>26 (87%)</td>
<td>19 (90%)</td>
<td>7 (78%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Co-channel blocker</td>
<td>15 (50%)</td>
<td>12 (57%)</td>
<td>3 (30%)</td>
<td>0.23</td>
</tr>
<tr>
<td>Proton pump inhibitor</td>
<td>7 (23%)</td>
<td>5 (24%)</td>
<td>2 (22%)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Defined as: under medical treatment or total cholesterol level >220 mg/dl or low-density cholesterol level >140 mg/dl; HbA1c >6.5%; systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg.

PCI, percutaneous coronary intervention; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II-receptor blocker.

Circulation Journal Vol. 73, February 2009
Definition of Clopidogrel Responsiveness

Classification of clopidogrel effectiveness was based on the definition from a previous report:IPA <10% (clopidogrel non-responders); 10% ≤ IPA <30% (hypo-responders); IPA ≥30% (responders).

Statistic Analysis

Continuous variables are expressed as mean±SD. Categorical variables are expressed as frequencies and percentages. Comparisons between categorical variables were performed using 2-tailed Fisher’s exact test or the Pearson’s chi-square test. Student’s t-test was used to compare continuous variables. Changes in parameters were analyzed using 1-sample t-test. A P-value <0.05 was defined as statistical significance. Statistical analyses were performed using StatView 5.0 software (SAS Institute, Cary, NC, USA).

Results

Characteristics of the Study Population

The baseline characteristics of the 30 enrolled patients are shown in Table 2. Mean age was 70±7 years and 22 patients (73%) were male. Only 1 patient (3%) had a history of prior myocardial infarction and 4 (13%) had undergone a prior PCI. Among the 30 patients, 9 did not undergo PCI because of unexpectedly mild stenosis on coronary angiography, which was not apparent on the initial non-invasive assessment. In the others (n=21), PCI with Cypher-stent implantation was successfully performed. Blood examination was performed until 48 h after intake of 300 mg clopidogrel for all 30 patients, and additionally, on days 14 and 28 post-procedure for the 21 patients undergoing PCI. Because some patients did not cooperate, and other administrative reasons, a few data points were not available. The number of patients evaluated for platelet function was as follows: at 4 h (n=29), 24 h (n=30), and 48 h (n=28) after clopidogrel intake (n=30), and at 14 days (n=20) and 28 days (n=21) among patients undergoing PCI (n=21) (Table 3). In all 30 enrolled patients, we did not observe any hematologic disorders or liver dysfunction during the study period.

Baseline characteristics were not significantly different between the PCI (n=21) and non-PCI (n=9) groups, apart from smoking habit (Table 2). As shown in Table 3, there was no significant difference between the 2 groups in the

---

Table 3 Maximal Aggregation Rates (%)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>14 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADP (5μmol/L stimulation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n=30)</td>
<td>64.5±5.7 (30)</td>
<td>53.8±10.1 (29)</td>
<td>52.9±8.9 (30)</td>
<td>49.0±10.2 (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCI-treated (n=21)</td>
<td>65.5±5.9 (21)</td>
<td>54.7±10.3 (20)</td>
<td>53.6±8.6 (21)</td>
<td>49.2±9.8 (20)</td>
<td>46.8±12.5 (20)</td>
<td>48.8±11.0 (21)</td>
</tr>
<tr>
<td>PCI-untreated (n=9)</td>
<td>62.0±8.2 (9)</td>
<td>52.0±10.0 (9)</td>
<td>52.0±10.0 (9)</td>
<td>48.2±11.8 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ADP (20μmol/L stimulation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n=30)</td>
<td>72.0±6.6 (28)</td>
<td>63.8±10.6 (27)</td>
<td>62.7±10.6 (28)</td>
<td>59.9±11.1 (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCI-treated (n=21)</td>
<td>72.8±6.5 (21)</td>
<td>63.8±10.7 (20)</td>
<td>62.7±9.6 (21)</td>
<td>60.1±11.4 (20)</td>
<td>55.8±10.2 (20)</td>
<td>57.0±10.3 (21)</td>
</tr>
<tr>
<td>PCI-untreated (n=9)</td>
<td>70.0±5.4 (9)</td>
<td>61.8±8.5 (9)</td>
<td>62.7±8.5 (9)</td>
<td>58.4±10.9 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Collagen (2μmol/L stimulation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n=30)</td>
<td>49.2±16.1 (28)</td>
<td>36.8±16.9 (27)</td>
<td>36.7±14.2 (28)</td>
<td>36.1±15.9 (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCI-treated (n=21)</td>
<td>50.2±15.8 (20)</td>
<td>40.1±16.8 (20)</td>
<td>38.3±13.2 (20)</td>
<td>37.7±14.2 (20)</td>
<td>33.1±12.2 (20)</td>
<td>34.8±11.9 (21)</td>
</tr>
<tr>
<td>PCI-untreated (n=9)</td>
<td>47.0±17.5 (9)</td>
<td>29.4±15.3 (9)</td>
<td>32.8±16.4 (9)</td>
<td>32.4±17.5 (8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation see in Table 2.
The number of examined subjects is shown in parentheses.

Fig 1. Time-dependent change in platelet aggregation after clopidogrel intake. (A) Maximal aggregation rates (MARs) induced with 5μmol/L ADP. (B) Inhibition of platelet aggregations (IPAs) with 5μmol/L ADP stimulation. (C) IPAs with 20μmol/L ADP stimulation; (D) IPAs with 2μg/ml collagen stimulation. By 1-sample t-test compared with the data at baseline, *P<0.0001.
ADP-induced MAR at baseline or at 4, 24 or 48 h. Therefore, both groups were analyzed together.

Platelet Aggregation
The MARs induced by 5 μmol/L ADP time-dependently decreased after clopidogrel intake (Fig 1A). The ADP values, which represent the degree of inhibition of platelet aggregability, increased reciprocally (Fig 1B). After 300-mg clopidogrel loading, rapid inhibition occurred at 4 h (IPA = 16.4±12.8%, P<0.0001 vs baseline), which continued until 24 h (IPA = 17.6±12.1%, P<0.0001 vs baseline). Following 75-mg clopidogrel intake, platelet aggregability was inhibited more intensely after 48 h (IPA = 24.0±13.9%, P<0.0001 vs 4 h and P<0.001 vs 24 h). It was noted that IPA did not attain a steady state within 24 h after the initial 300-mg clopidogrel intake. The same trend was observed with 20 μmol/L ADP (Fig 1C), for which the IPAs after clopidogrel intake were 11.9±13.6% at 4 h, 12.4±13.9% at 24 h, 16.3±16.3% at 48 h, 22.9±14.5% at 14 days, and 21.3±14.9% at 28 days. These data obtained with 5 or 20 μmol/L ADP stimulation suggest that clopidogrel efficiently exhibited antiplatelet effects and that a 300-mg loading dose might not be immediately sufficient to obtain the maximal antiplatelet effect.

Furthermore, clopidogrel intake also inhibited collagen-stimulated platelet aggregation (Fig 1D): IPAs after clopidogrel intake were 26.2±22.4% (4 h), 25.0±19.9% (24 h), 26.8±22.8% (48 h), 31.7±19.0% (14 days), and 29.5±24.9% (28 days).

Rates of Clopidogrel Responders and Non-Responders
We analyzed the inter-individual variation in 5 μmol/L ADP-induced platelet aggregability. Individual plots of the IPAs are shown in Fig 2. The effectiveness of clopidogrel exhibited a wide inter-individual variation and was quite constant in individual patients throughout the study period. The effects of clopidogrel were examined on the 14th and
28th days in 21 patients undergoing PCI. Among these patients, the IPAs at 48 h with 5 μmol/L ADP correlated well with those on the 14th day (P=0.04, r=0.49, n=18; Fig 3A) and the 28th day (P=0.0007, r=0.71, n=19; Fig 3B).

The proportion of responders, hypo-responders, and non-responders at 4 h with 5 μmol/L ADP was 7%, 69%, and 24%, respectively, and 36%, 50%, and 14%, respectively, at 48 h, indicating that the antiplatelet effects of clopidogrel at 48 h were stronger than those at 4 h, although we observed a rapid effect of clopidogrel at 4 h with the 300-mg loading dose. After 48 h, the antiplatelet effects of clopidogrel appeared to reach a plateau (Figs 1, 2). The rates of non-responders at 48 h, on the 14th day, and on the 28th day were 14%, 11%, and 20%, respectively, while the rates of responders were 36%, 47%, and 50% (Fig 3C).

Clopidogrel Responses Evaluated by VASP Phosphorylation

VASP is an abundant substrate of cAMP-dependent protein kinase in platelets. Binding of ADP to P2Y12 leads to Gi-coupled inhibition of adenylate cyclase, causing reduction of cAMP and the VASP-phosphorylation level in platelets. When P2Y12 receptors are successfully blocked by clopidogrel, the addition of ADP will not reduce the PGE1-induced VASP phosphorylation levels. Using these principles, VASP phosphorylation levels were evaluated by flow cytometry in the present study and the PRI was used to evaluate clopidogrel’s efficacy: the lower the PRI, the stronger the clopidogrel antiplatelet effect through inhibition of the P2Y12 receptor.

As shown in Fig 4, the PRIs gradually decreased after clopidogrel intake in a time-dependent manner: 70.2±19.0% at baseline, 62.9±20.4% at 4 h, 60.4±21.2% at 24 h, 52±20.0% at 48 h, 44.9±18.2% on day 14, and 43.8±23.9% on day 28. The PRIs and the IPAs at 48 h after clopidogrel intake were negatively correlated with each other (r=0.67).

Discussion

In this study, we evaluated the antiplatelet effect of clopidogrel under low-dose ASA therapy in Japanese patients scheduled for PCI, and found that there was a wide inter-individual variation and that the effects in Japanese may not be as strong as for Caucasians at the same dose.

We noted that the effectiveness of clopidogrel was reasonably constant in each patient throughout the study period (Fig 3), indicating that responsiveness is individual-specific. In a Western population, the rates of patients with so called ‘clopidogrel resistance’ ranged between 5% and 44%, although the definitions of clopidogrel resistance varied.6 As shown in Fig 3, we also detected 4 (14%) non-responders at 48 h in 1 patient (3%), clopidogrel suppressed ADP-induced platelet aggregability strongly at 4 h and throughout the study period. These data suggest that there is also a wide variety of responses to clopidogrel in the Japanese.

We used the definition of clopidogrel response proposed by Angiolillo et al because their study design was similar to ours, except that their patients took a higher dose of 250 mg ASA (vs 81–100 mg in our study) and platelet aggregation was evaluated with the optical aggregometer with 6 μmol/L ADP stimulation (vs 5 μmol/L ADP in our study)14. Therefore, the MAR at baseline in our study (64.5±19.0%) was equivalent to theirs (approximately 60–62%).14 Importantly, the ratio of responders at 4 h after a 300-mg loading dose was much lower in our study than in their study (7% vs 48%, respectively) and was also the case at 48 h, because the ratios of responders were 36% vs 80%, respectively. Another study conducted in Sweden demonstrated that the mean IPA with 20 μmol/L ADP was approximately 30% at 4 h after a 300-mg loading dose under 325 mg ASA therapy12 whereas the IPA with 20 μmol/L ADP in our study was 12%. Thus, the degree of platelet inhibition in the Japanese obtained with a similar regimen of clopidogrel, in which a 300-mg loading dose and 75-mg maintenance dose were administered under ASA therapy, might be lower than that in Western populations.

PRI values based on the VASP phosphorylation levels are becoming widely used for the evaluation of the antiplatelet effects of clopidogrel.16,17 We also found them useful because clopidogrel significantly inhibited the PRIs. Using the same loading/maintenance clopidogrel regimen, Grossmann et al reported that 10 (17.5%) of 57 patients were inadequate responders (PRI >50%) at 5 days.17 In the present study, the percentages of inadequate responders (PRI >50%) were 16/28 (57%) at 48 h, 10/20 (50%) at 14 days, and 7/21 (33%) at 28 days. Based on these results, we again consider that, at the present dosage, the antiplatelet effect of clopidogrel in the Japanese was not as strong as for Westerners.

Thus, on average, the antiplatelet effects of clopidogrel in Japanese patients are not as strong as those observed in Western people receiving a similar regimen of a 300-mg loading dose followed by a daily 75-mg maintenance dose under ASA therapy. To answer the question whether 75 mg/day clopidogrel is too strong for Japanese, we would answer that, based on the data presented here, it is not the case. Rather, the relatively weaker antiplatelet effect of clopidogrel in Japanese compared with in Western people might cause a higher incidence of stent thrombosis. However, currently we have no data on the degree of antiplatelet effect by clopidogrel that is necessary for the prevention of stent thrombosis in Japanese patients. Furthermore, because little data are available concerning the effect of ticlopidine in Japanese that would be sufficient to prevent stent thrombosis, we cannot conclude that the antiplatelet effect of clopidogrel at the current dosage is insufficient to prevent stent thrombosis. Further study is essential to link the effec-
tiveness of clopidogrel to the clinical outcomes of Japanese patients.

Our study clearly revealed that there are some clopidogrel non-responders among Japanese patients and thus their risk of stent thrombosis would be high. One possible solution could be to add cilostazol to the dual antiplatelet therapy of ASA and clopidogrel, because the functional mechanism of cilostazol, a phosphodiesterase 3 inhibitor, is partly similar to that of clopidogrel toward increasing the cAMP concentration in platelets\(^1\)
\(^{18}\) and its addition would enhance the antiplatelet effects of the dual antiplatelet therapy\(^1\)
\(^{19}\)
\(^{20}\)

The mechanisms of clopidogrel resistance are considered to involve both acquired and genetic factors.\(^2\)
Clopidogrel is a pro-drug, which needs to be activated to become the active substance through the action of Cyp3A4 and Cyp2C19. Single nucleotide polymorphisms (SNPs) in Cyp2C19 have been suggested as causes of resistance.\(^2\)
\(^{18}\) There is an inter-ethnic variability in the rate of the Cyp2C19 SNPs that cause Cyp2C19 to be non-functional and approximately 20% of Japanese people have been reported to possess little Cyp2C19 activity in contrast to only 2.5% of Westerners.\(^2\)
Therefore, a genetic defect in Cyp2C19 might have a great influence on clopidogrel effectiveness in the Japanese. Further examination is required.

Concomitant treatment with drugs metabolized by Cyp2C19 and Cyp3A4 might reduce the antiplatelet effect of clopidogrel. The proton-pump inhibitor, omeprazole, which is metabolized by Cyp2C19, has been reported to reduce clopidogrel efficacy.\(^2\)
In our study, only 3 patients were treated with omeprazole and their IPAs at 48 h using 5 \(\mu\)mol/L ADP were 6.0%, 14.5%, and 15.9%, respectively. Because the average IPA was 24.0±13.9% among 28 patients, the IPAs in the omeprazol-treated patients tended to be lower (\(P=0.12\), vs IPAs in omeprazole-free patients). A Cyp3A4 metabolizing drug, atorvastatin, has also been reported to affect clopidogrel’s efficacy,\(^2\)
\(^{22}\)\(^{24}\) although other reports showed no effects.\(^2\)
\(^{28}\)
\(^{29}\) In our study, IPA with 5 \(\mu\)mol/L ADP at 48 h was 21.2±10% (\(P=0.53\), vs IPAs in atorvastatin-free patients), suggesting that atorvastatin might not affect the antiplatelet effects of clopidogrel; however, our study was small-scale, so further study with a larger number of patients is essential for drawing conclusions concerning these drug interactions.

We observed a clear reduction of the collagen-induced platelet aggregrability by clopidogrel intake under dual antiplatelet therapy with ASA (Fig 1D). Collagen may induce aggregation mainly via the ADP pathway under ASA therapy. In other words, the signaling pathway stimulated by collagen might be shifted to the P2Y\(_12\) ADP-receptor pathway in platelets under ASA therapy, in which platelets cannot adequately generate thromboxane A\(_2\).

Evaluation of the antiplatelet effects of clopidogrel has been performed using several modalities, such as VerifyNow\(^3\)
\(^{30}\) and PFA100\(^3\)
\(^{31}\) both of which are whole-blood aggregometers, in addition to the optical aggregometer and analysis of VASP phosphorylation used in the present study. Further, the definition of clopidogrel resistance varies in each study. Thus, the method and definition used to evaluate the effect of clopidogrel have not yet been established, which would enable comparison of studies.

In summary, we showed that the antiplatelet effect of clopidogrel varied in Japanese patients, with 14% non-responders, and that, on average, the effect was not as strong as that observed in Western patients with a similar regimen of a 300-mg loading dose followed by a daily 75-mg main-

### Acknowledgements

We are grateful to all members in the Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University. This work was supported by Health and Labor Sciences Research Grant for Cardiovascular Research and a Grant from the Japan Cardiovascular Research Foundation.

### References


