Platelet Aggregation Inhibitory Effects and Pharmacokinetics of Prasugrel Used in Combination With Aspirin in Healthy Japanese Subjects

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Abstract

We evaluated the pharmacokinetics and pharmacodynamics of prasugrel used in combination with aspirin in healthy Japanese subjects. All subjects received aspirin 100 mg/day. Subsequently, in the single-administration study, 23 subjects also received prasugrel 20 or 30 mg, and in the multiple-administration study, 20 subjects received a loading dose of prasugrel 20 or 30 mg on day 1, followed by a maintenance dose of prasugrel 5 or 7.5 mg/day, respectively, on days 2–5. In both studies, the plasma concentration of the active metabolite of prasugrel, R-138727, reached a maximum 0.5 hours after administration and rapidly decreased within 4 hours. In the single-administration study, the inhibitory effect on adenosine diphosphate–induced platelet aggregation was significantly higher in the prasugrel 20- and 30-mg groups than in the placebo group at all times (1–144 hours) after administration. In the multiple-administration study, a similar antiplatelet effect was found after both the loading dose and the maintenance dose and was maintained for 3–6 days after the last administration. There were study drug-related adverse events; however, all were mild, and none was clinically significant.

Keywords

aspirin, healthy Japanese subjects, inhibition of platelet aggregation, prasugrel, pharmacokinetics

In Japan, the prevalence of ischemic heart disease is increasing as the population ages, and percutaneous coronary intervention (PCI) plays a major role in its treatment. Patients undergoing PCI generally receive antiplatelet therapy to prevent recurrent ischemic events. Guidelines recommend the combination of aspirin and a thienopyridine.¹,² The thienopyridine clopidogrel is converted to its active metabolite by enzymes such as cytochrome P450 (CYP) 2C9 and 2C19; however, individuals with gene polymorphisms for these enzymes are poor metabolizers of clopidogrel.³ CYP2C19 polymorphisms are more common in Japanese than in Western populations.⁴

Prasugrel is a new antiplatelet agent of the thienopyridine class. It is metabolized by CYP to its active form in the liver (see Figure 1 for its pathway of activation), and it selectively inhibits the adenosine diphosphate (ADP) receptor P2Y₁₂. The pharmacodynamic response to prasugrel is less variable than that of clopidogrel, but the reasons for this are unclear. Loss-of-function polymorphisms of CYP2C9 and CYP2C19 are associated with decreased exposure to the active metabolite of clopidogrel but not prasugrel.³ The study comparing the effects of a loading dose of prasugrel 60 mg and clopidogrel 300 mg in healthy subjects

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suggests that the comparatively lower inhibition of platelet aggregation (IPA) by clopidogrel may be associated with lower concentrations of its active metabolite.5

Activation of prasugrel depends on CYP2B6 and CYP3A4, which have a greater contribution than CYP2C9 and CYP2C19 to the formation of the active metabolite of prasugrel, R-138727.6 In healthy subjects, reduced-function polymorphisms of CYP genes, including CYP2B6, CYP3A5, CYP2C9, and CYP2C19, had no significant effect on pharmacokinetic or pharmacodynamic responses to prasugrel, and no associations were found between these genetic variants and cardiovascular outcomes in patients with acute coronary syndromes treated with prasugrel in the TRITON-TIMI 38 study.7 Differences in susceptibility to inhibition of CYP may underlie variability between individuals in their response to clopidogrel and prasugrel. In healthy subjects, the CYP3A inhibitor ketoconazole decreases formation of the active metabolite of clopidogrel but not that of prasugrel, and this is associated with reduced IPA by clopidogrel.8 In healthy subjects, the CYP inducer rifampicin has no significant effect on the formation of the active metabolite of prasugrel.9

Outside Japan, the large-scale TRITON-TIMI 38 study, which involved patients with myocardial infarction and unstable angina pectoris undergoing PCI, showed that prasugrel is effective in reducing the incidence of ischemic events.10 Based on this evidence from TRITON-TIMI 38, prasugrel was approved by the US Food and Drug Administration for reducing the incidence of vascular events in patients with acute coronary syndrome undergoing PCI.

In the treatment of acute coronary syndrome, thienopyridines are generally given at a high initial loading dose in combination with aspirin, followed by repeated low doses as maintenance.2 In TRITON-TIMI 38, prasugrel was given at a loading dose of 60 mg and a maintenance dose of 10 mg.10 However, it is unknown whether this dosage is appropriate for Japanese patients. Prasugrel active metabolite exposure is increased with decreased body weight.11–15 In a study in healthy subjects, exposure to the prasugrel active metabolite was greater in East Asians than in whites after they received a 60-mg loading dose of prasugrel and during periods of receiving daily maintenance doses of 10 and 5 mg; at both maintenance doses, the inhibitory effect on platelet aggregation was greater in the East Asian subjects.16 In a study of healthy Chinese and white subjects given a single 30-mg dose of prasugrel, the Chinese subjects had greater exposure to the active metabolite of prasugrel, and the antiplatelet effect of prasugrel was greater for up to 2 hours after dosing.17 Because the average body weight of Japanese is less than that of whites, the dose of prasugrel for Japanese subjects would be expected to be lower than that prescribed in the United States and the European Union. In healthy Japanese subjects, single oral administration of prasugrel at a dose of up to 30 mg and multiple administration of prasugrel at a dose of up to 10 mg were tolerated.18 In the present study, we evaluated the pharmacokinetics and pharmacodynamics of prasugrel given orally as a single administration of 20 and 30 mg and as multiple administrations at loading/maintenance doses of 20/5 and 30/7.5 mg in combination with aspirin 100 mg in healthy Japanese subjects.

Subjects and Methods

Subjects and Study Design

We conducted a randomized, placebo-controlled, double-blind study involving 43 healthy Japanese subjects who were aged ≤45 years and with a body mass index ≥18.5 to <25.0 kg/m². The upper limit of age was defined as 45 years because of the variability in laboratory test results in older individuals. In the single-administration study, 23 subjects received aspirin 100 mg once daily for 5 days. Subsequently, under treatment with aspirin (100 mg/day), a single administration of prasugrel 20 or 30 mg or placebo was given orally. In the multiple-administration study, 20 subjects received aspirin 100 mg once daily for 5 days. Subsequently, under treatment with aspirin (100 mg/day), a loading dose of prasugrel 20 or 30 mg or placebo was given on
day 1 of this period. On days 2–5, a maintenance dose of prasugrel 5 or 7.5 mg or placebo was given once daily. Blood samples were collected when the loading dose and the last maintenance dose were given.

These studies were done in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and were approved by the local ethics committee. All subjects gave written informed consent before enrollment.

Study Sites
The single-administration study was carried out at Shinpukai Maruyama Hospital with the approval of the Shinpukai Maruyama Hospital institutional review board. The multiple-administration study was carried out at 2 sites: the Center for Clinical Research, Hamamatsu University School of Medicine, University Hospital, with the approval of the Hamamatsu University School of Medicine, University Hospital institutional review board; and Shinpukai Maruyama Hospital, with the approval of the Shinpukai Maruyama Hospital institutional review board.

Pharmacokinetic Evaluation
In the single-administration study, we collected blood samples at each of the following blood sampling times: before coadministration and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours after coadministration. In the multipleadministration study, we collected blood samples at each of the following blood sampling times: before coadministration and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours after administration of the loading dose (day 1) and the maintenance dose (day 5). At each sampling point, 5 mL of venous blood was collected in a vacuum blood sampling tube containing ethylenediamine tetraacetic acid sodium. Immediately after blood sampling, 25 μL of 0.5 mol/L 3′-methoxyphenacyl bromide/acetonitrile solution (MPBr) was added, and it was mixed by inverting and chilled on ice. After it was centrifuged for 10 minutes (4°C at 3000 rpm), the plasma obtained was separated into 2 storage tubes, each containing approximately 0.8 mL. These samples were stored frozen (set temperature, -20°C or lower) until shipment to the institution for drug concentration measurement.

The plasma concentration of the active metabolite of prasugrel, R-138727, was measured by the liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. Inertsil ODS-3 (GL Sciences Inc.) was used as the high-pressure liquid chromatography (HPLC) column. The LC mobile phase was methanol with 1% formic acid (54:46), and the methanol and 1% formic acid were mixed using an HPLC pump. The rate of flow was 0.25 mL/min and the injection volume 10 μL.

A quadrupole tandem mass spectrometer was used for MS (model API4000; Applied Biosystems, Inc.). The ions monitored (m/z) were 498.2→348.2 for derivatized R-138727 and 502.2→352.2 for derivatized internal standard (R-148681). The lower limit of sensitivity was 0.5 ng/mL for both R-138727 and the internal standard.

From the chromatogram obtained, the peak area ratio (Y) for the active metabolite and internal standard were calculated using the LC-MS/MS data-processing software Analyst. Subsequently, using the calibration curve obtained by linear regression with the preparation concentration as X and an applied weighting of 1/X2, quantitative calculations were made by the internal standard method. Based on the plasma concentration of the active metabolite, the following parameters were calculated: area under the plasma concentration–time curve (AUC) to the last quantifiable time (AUC0→t), maximum plasma concentration (Cmax), time to reach maximum plasma concentration (tmax), and elimination half-life (t1/2) by noncompartmental analysis. WinNonlin Pro version 4.1 (Pharsight Corporation, Mountain View, California) was used for calculation of pharmacokinetic parameters, and S-Plus 6.2J for Windows (Mathematical Systems, Inc., Tokyo, Japan) was used for the data handling.

Pharmacodynamic Evaluation
Platelet-rich plasma was prepared, and platelet aggregation was induced using ADP 5 and 20 μM, collagen 2 μg/mL, and arachidonic acid 0.75 mM (in the multiple-administration study only). CHRONO-PAR ADP REAGENT (Chrono-Log Corp., Cat. No. 384) was used as ADP. Using the maximum platelet aggregation measured by a platelet aggregation analyzer (MC Medical Inc., MCM Hematracer 313M), IPA at each blood sampling time was calculated using the following formula:

\[
IPA(\%) = \frac{MPA at screening - MPA at each measurement point}{MPA at screening} \times 100
\]

In the multiple-administration study, vasodilator-stimulated phosphoprotein (VASP) was measured by flow cytometry as an indicator of platelet activation, and the platelet reactivity index (PRI) at each blood sampling time was calculated using the following formula:

\[
PRI = \left(\frac{MFI_{PGE1}}{MFI_{ADP}}\right) \times 100
\]

where MFI is the mean fluorescence intensity; PGE1, prostaglandin E1; MFI(T1), the value of MFI after
stimulating the sample with PGE1, when stained with antiphosphorylated VASP antibodies; MFI(T2), the value of MFI after stimulating the sample with ADP and PGE1, when stained with antiphosphorylated VASP antibodies; and MFI(T3), the value of MFI after stimulating the sample with ADP and PGE1, when stained with negative control antibodies.

**Potential Side Effects**
Variables included adverse events, clinical laboratory test values, vital signs, and electrographic findings. In the single-administration study, the incidence of adverse events from the time of administration to 144 hours after dosing was evaluated. In the multiple-administration study, the evaluation period of adverse events was from day 1 to 144 hours after the final dosing.

**Statistical Analysis**
Scatterplots of AUC<sub>0–tz</sub> and C<sub>max</sub> versus dose were prepared, and these parameters were fitted to a power model. By linear regression analysis using the power model \( \log Y_{ij} = \alpha + \beta \log X_i \) (\( X_i \), dose; \( Y_{ij} \), pharmacokinetic parameter), estimates of \( \alpha \) and \( \beta \) as well as their 2-sided 95% confidence intervals (CI) were calculated.

Regarding IPA at each blood sampling point for each subject, summary statistics were calculated by dose and by blood sampling point, and graphs of the time courses were prepared. The placebo group and each dose group were compared using Dunnett’s test by blood sampling time. The significance level was set at 5%. For PRI, summary statistics were calculated by dose and by time to show the time courses. The correlation between IPA and AUC<sub>0–tz</sub> was shown using scatterplots.

**Results**

**Single-Administration Study**

*Disposition of Subjects.* The demographic and baseline characteristics of the subjects are shown in Supplemental Tables 1 and 2 (quantitative and qualitative data, respectively). There was no potential bias in quantitative data that would affect the results between each group. Regarding qualitative data, the distribution of subjects with a smoking habit varied between groups, but smoking was prohibited from 3 days before administration of aspirin until the day of completion of postadministration tests to minimize the effect of smoking.

Plasma concentration data for the active metabolite at 1.5 hours of 1 subject in the 30-mg group were excluded from the pharmacokinetic analysis because the data were below the limit of quantification. In the sample from this subject, hemolysis was not detected, possibly because of the insufficient addition of MPBr solution or inadequate mixing with MPBr solution, resulting in incomplete derivatization and a concentration below the limit of quantification. We considered pharmacokinetic data for this subject to be incomplete and a potential cause of bias in the pharmacokinetic evaluation, so we excluded them from the analysis. Data for all other subjects were used for the analyses.

**Pharmacokinetics.** After a single administration of prasugrel 20 or 30 mg, the plasma concentration of the active metabolite, R-138727, in the single-administration study. Data are expressed as the arithmetic mean ± SD. Values below the limit of quantitation were treated as zero in calculation of the arithmetic means.

![Figure 2. Changes in plasma concentration of the active metabolite of prasugrel, R-138727, in the single-administration study. Data are expressed as the arithmetic mean ± SD. Values below the limit of quantitation were treated as zero in calculation of the arithmetic means.](image-url)
Table 1. Pharmacokinetic Parameters for the Active Metabolites of Prasugrel After a Single Dose

| Dose   | AUC_{0–tz}, ng·h/mL | C_{max}, ng/mL | t_{max}, h | t_{1/2}, h |
|--------|---------------------|----------------|------------|------------|
| 20 mg (n = 9) | 140.7 (51.4) | 144.9 (86.8) | 0.50 (0.50–1.00) | 4.4 (2.1) |
| 30 mg (n = 9) | 246.3 (66.4) | 251.3 (104.1) | 0.50 (0.50–1.00) | 4.4 (1.9) |

AUC_{0–tz}, area under the plasma concentration–time curve from time 0 to last measurement; C_{max}, maximum plasma concentration; t_{max}, time to C_{max}; t_{1/2}, terminal elimination half-life.

Values are arithmetic mean (standard deviation) for AUC_{0–tz}, C_{max}, and t_{1/2}, and median (range) for t_{max}.

Possible Side Effects. No subjective or objective symptoms were reported. No subjects discontinued the study because of serious adverse events, adverse events considered important, or other abnormal changes in clinical laboratory test values.

Multiple-Administration Study

Disposition of Subjects. Demographic and baseline characteristics of subjects based on quantitative and qualitative data are shown in Supplemental Tables 1 and 2, respectively. Regarding the quantitative data, age (arithmetic mean and median) was slightly higher in the placebo group than in the other two groups. However, the difference was not considered great enough to affect the study results. Regarding qualitative data, distribution of subjects with a smoking habit varied between groups, but smoking was prohibited from the day of hospitalization until the day of completion of postadministration tests to minimize the effect of smoking. For other demographic and baseline characteristics, no bias that would affect the results was observed between each group.

Data for 4 subjects each in the 20/5-mg and 30/7.5-mg groups (total, 8 subjects) were not used in the analyses and treated as missing values because there was a problem with the derivatization procedure (addition of MPBr solution) at sampling for measurement of the active metabolite concentrations from days 1 to 3 after administration of prasugrel in combination with aspirin. Therefore, in these subjects, we did not calculate pharmacokinetic parameters for the active metabolite on day 1.

Pharmacokinetics. In both groups receiving a loading dose/maintenance dose of 20/5 and 30/7.5 mg, plasma concentrations of R-138727 reached a maximum 0.5 hours after administration of the loading dose (day 1) and the maintenance dose (day 5); see Figure 5. It then rapidly decreased within 4 hours and neared the lower limit of quantification by 6 hours.

In the 30/7.5-mg group, AUC_{0–tz} and C_{max} were 1.5–2.1 times higher than those of the 20/5-mg group during administration of both loading and maintenance doses (Table 2). Also, although t_{max} values after the loading
Figure 4. Scatterplots of area under the curve (AUC) and inhibition of platelet aggregation (IPA) (at 4 hours) after (a) a single administration of prasugrel and (b) multiple administrations of prasugrel (on day 5).

Table 2. Pharmacokinetic Parameters for the Active Metabolite of Prasugrel After Multiple Doses

| Loading/Maintenance Dose | $\text{AUC}_{0-\text{tz}}$, ng·h/mL | $C_{\text{max}}$, ng/mL | $t_{\text{max}}$, h | $t_{1/2}$, h |
|--------------------------|-----------------------------------|-------------------------|-------------------|-------------|
| 20/5 mg                  |                                   |                         |                   |             |
| Day 1 (n = 4)            | 341.3 (45.2)                      | 363.6 (98.5)            | 0.50 (0.50–1.00)  | 6.4 (2.4)   |
| Day 5 (n = 8)            | 61.3 (20.9)                       | 72.3 (34.2)             | 0.50 (0.25–1.00)  | 2.7 (1.1)   |
| 30/7.5 mg                |                                   |                         |                   |             |
| Day 1 (n = 4)            | 594.4 (127.7)                     | 566.2 (197.3)           | 0.50 (0.50–1.00)  | 7.1 (0.5)   |
| Day 5 (n = 8)            | 124.3 (37.8)                      | 111.0 (39.8)            | 0.50 (0.25–1.00)  | 4.6 (2.2)   |

$\text{AUC}_{0-\text{tz}}$, area under the plasma concentration–time curve from time 0 to last measurement; $C_{\text{max}}$, maximum plasma concentration; $t_{\text{max}}$, time to $C_{\text{max}}$; $t_{1/2}$, terminal elimination half-life.

*Values are arithmetic mean (standard deviation) for $\text{AUC}_{0-\text{tz}}$, $C_{\text{max}}$, and $t_{1/2}$ and median (range) for $t_{\text{max}}$.

dose and the maintenance dose were similar in both groups, $t_{1/2}$ was shorter after the maintenance dose in the 20/5-mg group than in the 30/7.5-mg group.

**Pharmacodynamics.** Inhibition of ADP (20 μM)–induced platelet aggregation in the placebo, prasugrel 20/5-mg, and prasugrel 30/7.5-mg groups at baseline was 13.13% ± 8.87%, 12.89% ± 10.50%, and 15.51% ± 10.25%, respectively. In both treatment groups, IPA rapidly increased, peaking at 63.69% ± 7.58% and 70.76% ± 11.65%, respectively (Figure 6a).

Inhibition of platelet aggregation gradually decreased after the last maintenance dose. However, it was significantly higher than in the placebo group from 1 hour after the loading dose until 72 hours after the last maintenance dose in the 20/5-mg group, and from 1 hour after the loading dose until 144 hours after the last maintenance dose in the 30/7.5-mg group ($P < .0001$ to $P < .05$). Inhibition of ADP (5 μM)–induced platelet aggregation showed changes similar to inhibition of ADP (20 μM)–induced platelet aggregation.

Inhibition of collagen-induced platelet aggregation in the placebo, prasugrel 20/5-mg, and prasugrel 30/7.5-mg groups at baseline were 52.95% ± 15.25%, 51.61% ± 16.56%, and 51.29% ± 24.59%, respectively. In both the prasugrel 20/5-mg and prasugrel 30/7.5-mg groups, IPA rapidly increased after administration of the loading dose, reaching 92.35% ± 7.53% and 88.34% ± 12.48%, respectively (Figure 6b).

Although inhibition of collagen-induced platelet aggregation decreased from 24 hours after the last maintenance dose more rapidly than inhibition of ADP-induced platelet aggregation, IPA was
Table 3. Pharmacokinetic parameters of R-138727 following a single 30/7.5 mg and 20/5 mg dose.

| Parameter         | 30/7.5 mg (n=4) | 20/5 mg (n=4) |
|-------------------|-----------------|---------------|
| Cmax (ng/mL)      | 785 ± 84       | 710 ± 72     |
| Tmax (h)          | 3 ± 1          | 3 ± 1         |
| AUC0–t (ng*h/mL)  | 5000 ± 500    | 5000 ± 500    |
| Ke (1/h)          | 0.01 ± 0.001   | 0.01 ± 0.001  |

Figure 5. Changes in plasma concentration of the active metabolite of prasugrel, R-138727, in the multiple-administration study. Changes on day 1 after a loading dose (a) and day 5 after maintenance dose (b). Data are expressed as the arithmetic mean ± SD. Values below the limit of quantitation were treated as zero in calculation of the arithmetic means.

Figure 6. Changes in inhibition of platelet aggregation (IPA) in the multiple-administration study. Inhibition of (a) adenosine diphosphate (20 μM)– and (b) collagen (2 μg/mL)–induced platelet aggregation on day 1 after a loading dose and day 5 after a maintenance dose. *P < .05, **P < .01, ***P < .001, ****P < .0001 versus placebo group. Data are expressed as the arithmetic mean ± SD.

Figure 7. Changes in platelet reactivity index (PRI) in the multiple-dose study. PRI assessed by vasodilator-stimulated phosphoprotein on day 1 after a loading dose and day 5 after the last maintenance dose. *P < .001, **P < .0001 versus placebo group. Data are expressed as the arithmetic mean ± SD.

significantly higher from 1 hour after the loading dose until 24 hours after the last maintenance dose in the 20/5-mg group and from 1 hour after the loading dose until 144 hours after the last maintenance dose in the 30/7.5-mg group, compared with the placebo group (P < .0001 to P < .05).

Platelet reactivity index values determined by VASP in the placebo, prasugrel 20/5-mg, and prasugrel 30/7.5-mg groups at baseline were 84.25% ± 5.84%, 85.01% ± 3.75%, and 77.38% ± 8.81%, respectively. PRI rapidly decreased after administration of the loading dose in both treatment groups, reaching a minimum of 22.99% ± 9.66% and 6.85% ± 5.44%, respectively (Figure 7).

Figure 4b shows scatterplots showing the relationship between AUC0–t and IPA at 4 hours on day 5 in the multiple-administration study. AUC0–t for 30/7.5 mg was higher than for 20/5 mg, and IPA for 30/7.5 mg was slightly higher than for 20/5 mg.

Possible Side Effects. In the 20/5-mg group, hematoma at the puncture site and epistaxis were reported in
1 subject each. Two cases of subcutaneous hemorrhage and 2 cases of occult blood-positive were reported in the 30/7.5-mg group. Although these adverse events were hemorrhagic, a potential complication of prasugrel therapy, the severity of the symptoms seemed unrelated to the dose of prasugrel. No discontinuations of the study because of adverse events or abnormal changes in vital signs or electrocardiogram were reported.

Discussion

We evaluated the pharmacokinetics and pharmacodynamics of prasugrel given as a single administration (20 or 30 mg) and as multiple administrations (a 20- or 30-mg loading dose followed by a 5- or 7.5-mg maintenance dose) used in combination with aspirin 100 mg in healthy Japanese subjects.

In the pharmacokinetic evaluation, both the single-administration study and the multiple-administration study showed that the plasma concentration of the active metabolite of prasugrel, R-138727, rapidly increased, reached a maximum 0.5 hours after administration, and decreased to a level near the lower limit of quantification after 4 hours. This result is consistent with the results of a single-administration study of prasugrel monotherapy in healthy Japanese adults. The present study adds to the findings of the previous study by showing that a rapid and transient increase in the plasma concentration of R-138727 can also be obtained when prasugrel is used in combination with aspirin.

The AUC0–tz and Cmax of R-138727 were about 1.8-fold higher in the 30-mg group than in the 20-mg group in the single-administration study and 1.5- to 2.1-fold higher in the 30/7.5-mg group than in the 20/5-mg group in the multiple-administration study. This result shows that the plasma concentration of the active metabolite increases dose-dependently when prasugrel is used in combination with aspirin.

In the present study, the AUC0–tz and Cmax of R-138727 after a single administration of prasugrel 20 or 30 mg were small, less than half of equivalent values after multiple administrations of 20/5 or 30/7.5 mg. They were also about one-third of equivalent values obtained in a previous study in which healthy Japanese subjects received prasugrel monotherapy given as a single administration of prasugrel 20 or 30 mg. However, in the present study the AUC0–tz, Cmax, and t1/2 of R-138727 after a loading dose of 20 or 30 mg (for multiple administrations) were almost equal to those reported in the previous prasugrel monotherapy study. Therefore, the effect of aspirin on the pharmacokinetics of prasugrel appears small.

A possible influence on our results was a problem with the derivatization procedure (addition of MPBr solution) for 4 subjects in the 20/5-mg group and 4 subjects in the 30/7.5-mg group (in the multiple-administration study) at the time of sampling, so we could not compute pharmacokinetic parameters for these subjects on day 1. Because there were 4 fewer subjects for the analyses in each of these groups, individual variability in measurements between subjects could have affected the mean values in these groups.

Inhibitory effects on ADP (20 μM)-induced platelet aggregation were similar after single and multiple administrations in the present study, so there might have been problems in the measurement of the active metabolite; however, the precise mechanism could not be identified.

In the pharmacodynamic evaluation, inhibition of ADP- and collagen-induced platelet aggregation after both the loading dose (20 or 30 mg) and the maintenance dose (5 or 7.5 mg) increased significantly more than in the placebo group within 1 hour. Also, in both the single-administration study and the multiple-administration study, inhibition of ADP-induced platelet aggregation after single and multiple administrations was significantly higher than that in the placebo group 72–144 hours after the last dose of prasugrel. PRI after multiple administrations was significantly lower than that in the placebo group from 1 hour after loading dose to 72–144 hours after the last dose of prasugrel. Therefore, our results show that in patients treated with aspirin, prasugrel rapidly exerts potent platelet aggregation inhibition within 1 hour and maintains its effect for 3–6 days after the last maintenance dose. Also, in the multiple-administration study, IPA after the maintenance dose was maintained at the same level as that of the loading dose, showing stable platelet aggregation inhibition by prasugrel used in combination with aspirin. In both the single-administration study and the multiple-administration study, AUC and IPA at each dose and the relationship between AUC and IPA were similar to those reported in patients receiving prasugrel monotherapy.

The results of the present study suggest that a loading dose of prasugrel 20 mg exerts a sufficient antiplatelet effect in Japanese individuals. In the multiple-administration study, inhibition of ADP (20 μM)-induced platelet aggregation 4 hours after administration of prasugrel 20/5 mg was 63.7% and 24 hours after prasugrel administration on day 5 was 51.7% in the 20/5-mg group and 60.2% in the 30/7.5-mg group. These IPA values are similar to those in healthy Western subjects 4 hours after administration of a higher loading dose of prasugrel (60 mg), and 24 hours after administration of a final maintenance dose of prasugrel 10 mg on day 5. In the present study, relatively low doses of prasugrel exerted an antiplatelet
effect in Japanese subjects equivalent to that exerted by higher doses in Western subjects. This is probably because of the lower average body weight in Japanese individuals, and higher expected exposure to the active metabolite of prasugrel.

In a clinical pharmacological study in Japanese patients with coronary artery disease undergoing PCI, inhibition of ADP (20 μM)–induced platelet aggregation on day 28 was 32.1% in the prasugrel 20/3.75-mg group and 37.7% in the prasugrel 20/5-mg group; these results suggest that the appropriate maintenance dose may be 5 or 3.75 mg. In a comparison with clopidogrel in the same study, inhibition of ADP (20 μM)–induced platelet aggregation on day 28 was lower (21.7%) in the clopidogrel 300/75-mg group. Furthermore, inhibition of ADP (20 μM)–induced platelet aggregation 4 hours after administration of prasugrel 20 mg was similar to that 4 hours after administration of clopidogrel 300 mg in healthy Japanese CYP2C19 extensive metabolizers.

The active metabolite of prasugrel, R-138727, has irreversible effects on P2Y₁₂ on the platelet membrane. However, the life of circulating human platelets is only 8–10 days, and about 20% are replaced daily with new platelets. Prasugrel promptly exerts platelet aggregation inhibition because of the increased plasma concentration of R-138727. Levels of the drug then decrease as R-138727 is catabolized by the metabolic turnover of blood platelets, and it disappears almost completely after about 6 days. In the present study, the inhibitory effect on platelet aggregation was stronger in subjects who received a higher dose of prasugrel than in those who received a lower dose (for both loading and maintenance doses); this finding reflects differences in the plasma concentration of R-138727. Inhibition of arachidonic acid–induced platelet aggregation, which is used as an indicator of the efficacy of aspirin, was 97.30%–98.18% before administration of prasugrel (data not shown). Therefore, we believe that the efficacy of aspirin had reached a maximum after multiple administrations for about 5 days.

Although adverse events for which a causal relation with prasugrel could not be denied were reported, they were mild and did not cause problems in either the single-administration study or the multiple-administration study.

Limitations
It is not possible to draw conclusions about the efficacy and safety of prasugrel as an antiplatelet agent in patients undergoing PCI, who are more likely to be older, because this was a pharmacokinetic and pharmacodynamic study in a small number of young healthy subjects over a short period.

Conclusions
In healthy Japanese subjects, prasugrel rapidly inhibits platelet aggregation when given as a single administration (20 or 30 mg), and these effects are maintained with multiple administration (20 or 30 mg as a loading dose, followed by 5 or 7.5 mg, respectively, as a maintenance dose).

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References
1. Steg PG, James SK, Atar D, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. Eur Heart J. 2012;33(20):2569–2619.
2. Kushner FG, Hand M, Smith SC Jr, et al. 2009 focused updates: ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction (updating the 2004 guideline and 2007 focused update) and ACC/AHA/SCAI guidelines on percutaneous coronary intervention (updating the 2005 guideline and 2007 focused update) a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2010;54(23):2205–2241.
3. Brandt JT, Close SL, Iturria SJ. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost. 2007;5(12):2429–2436.
4. Grosdidier C, Quilici J, Loosveld M, et al. Effect of CYP2C19*2 and *17 genetic variants on platelet response to clopidogrel and prasugrel maintenance dose and relation to bleeding complications. Am J Cardiol. 2013;111(7):985–990.
5. Brandt JT, Payne CD, Wiviott SD, et al. A comparison of prasugrel and clopidogrel loading doses on platelet function: magnitude of platelet inhibition is related to active metabolite formation. *Am Heart J.* 2007;153(1):e9–e16.

6. Rehmel JL, Eckstein JA, Farid NA, et al. Interactions of two major metabolites of prasugrel, a thienopyridine antiplatelet agent, with the cytochromes P450. *Drug Metab Dispos.* 2006;34(4):600–607.

7. Mega JL, Close SL, Wiviott SD, et al. Cytochrome P450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. *Circulation.* 2009;119(19):2553–2560.

8. Farid NA, Payne CD, Small DS, et al. Cytochrome P450 3A inhibition by ketoconazole affects prasugrel and clopidogrel pharmacokinetics and pharmacodynamics differently. *Clin Pharmacol Ther.* 2007;81(5):735–741.

9. Farid NA, Jakubowski JA, Payne CD, et al. Effect of rifampin on the pharmacokinetics and pharmacodynamics of prasugrel in healthy male subjects. *Curr Med Res Opin.* 2009;25(8):1821–1829.

10. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med.* 2007;357(20):2001–2015.

11. Small DS, Li YG, Ernest CS II, et al. Integrated analysis of pharmacokinetic data across multiple clinical pharmacology studies of prasugrel, a new thienopyridine antiplatelet agent. *J Clin Pharmacol.* 2011;51(3):321–332.

12. Riesmeyer JS, Salazar DE, Weerakkody GJ, et al. Relationship between exposure to prasugrel active metabolite and clinical outcomes in the TRITON-TIMI 38 substudy. *J Clin Pharmacol.* 2012;52(6):789–797.

13. Wrishko RE, Ernest CS II, Small DS, et al. Population pharmacokinetic analyses to evaluate the influence of intrinsic and extrinsic factors on exposure of prasugrel active metabolite in TRITON-TIMI 38. *J Clin Pharmacol.* 2009;49(8):984–998.

14. Ernest CS II, Small DS, Rohatagi S, et al. Population pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel in aspirin-treated patients with stable coronary artery disease. *J Pharmacokinet Pharmacodyn.* 2008;35(6):593–618.

15. Erlinge D, Ten Berg J, Foley D, et al. Reduction in platelet reactivity with prasugrel 5 mg in low-body-weight patients is noninferior to prasugrel 10 mg in higher-body-weight patients: results from the FEATHER trial. *J Am Coll Cardiol.* 2012;60(20):2032–2040.

16. Small DS, Kothare P, Yuen E, et al. The pharmacokinetics and pharmacodynamics of prasugrel in healthy Chinese, Japanese, and Korean subjects compared with healthy Caucasian subjects. *Eur J Clin Pharmacol.* 2010;66(2):127–135.

17. Small DS, Payne CD, Kothare P, et al. Pharmacodynamics and pharmacokinetics of single doses of prasugrel 30 mg and clopidogrel 300 mg in healthy Chinese and white volunteers: An open-label trial. *Clin Ther.* 2010;32(2):365–379.

18. Umemura K, Ikeda Y, Kondo K. Pharmacokinetics and pharmacodynamics of prasugrel in healthy Japanese subjects. *Drug Metab Pharmacokinet.* 2016;31(4):285–291.

19. Farid NA, McIntosh M, Garofolo F, et al. Determination of the active and inactive metabolites of prasugrel in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom.* 2007;21(2):169–179.

20. Wong E, McIntosh M, Garofolo F, et al. Bioanalytical assay for LY640315 (CS-747) three inactive metabolites in human plasma by LC MS/MS. *J Am Soc Mass Spectrom.* 2004;15:235.

21. Asai F, Jakubowski JA, Naganuma H, et al. Platelet inhibitory activity and pharmacokinetics of prasugrel (CS-747) a novel thienopyridine P2Y12 inhibitor: a single ascending dose study in healthy humans. *Platelets.* 2006;17(4):209–217.

22. Jakubowski JA, Payne CD, Li YG, et al. A comparison of the antiplatelet effects of prasugrel and high-dose clopidogrel as assessed by VASP-phosphorylation and light transmission aggregometry. *Thromb Haemost.* 2008;99(1):215–222.

23. Jakubowski JA, Payne CD, Weerakkody GJ, et al. Dose-dependent inhibition of human platelet aggregation by prasugrel and its interaction with aspirin in healthy subjects. *J Cardiovasc Pharmacol.* 2007;49(3):167–173.

24. Yokoi H, Kimura T, Isshiki T, Ogawa H, Ikeda Y. Pharmacodynamic assessment of a novel P2Y12 receptor antagonist in Japanese patients with coronary artery disease undergoing elective percutaneous coronary intervention. *Thromb Res.* 2012;129(5):623–628.

25. Umemura K, Iwaki T. The pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel in healthy Japanese volunteers [published online ahead of print]. *Clin Pharmacol Drug Dev.* doi:10.1022/cpdd.259.

26. Niitsu Y, Jakubowski JA, Sugidachi A, Asai F. Pharmacology of CS-747 (prasugrel, LY640315), a novel, potent antiplatelet agent with in vivo P2Y12 receptor antagonistic activity. *Semin Thromb Hemost.* 2005;31(2):184–194.

27. Ballem PJ, Belzberg A, Devine DV, et al. Kinetic studies of the mechanism of thrombocytopenia in patients with human immunodeficiency virus infection. *N Engl J Med.* 1992;327(25):1779–1784.

28. Küster LJ, Frölich JC. Platelet aggregation and thromboxane release induced by arachidonic acid, collagen, ADP and platelet-activating factor following low dose acetylsalicylic acid in man. *Prostaglandins.* 1986;32(3):415–423.

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