Pharmacodynamic Comparisons for Single Loading Doses of Prasugrel (30 mg) and Clopidogrel (600 mg) in Healthy Korean Volunteers
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Background: Previous studies involving a loading dose (LD) of 60 mg prasugrel have suggested that active metabolite exposure and pharmacodynamic responses may be higher in persons of Asian ethnicity than in Caucasian subjects. The aim of this study was to determine the pharmacodynamic effect of an LD of 30 mg prasugrel and 600 mg clopidogrel in healthy Korean volunteers.

Methods and Results: Twelve volunteers were randomly assigned to a prasugrel or a clopidogrel group. Following a 2-week washout period, group designations and treatments were switched (6 per group). Platelet function was serially measured via light transmission aggregometry (LTA), VerifyNow and multiple electrode platelet aggregometry (MEA) assays at baseline and 0.5, 2, 6, and 24 h after LD. Inhibition of platelet aggregation (IPA) at 0.5–24 h after prasugrel was significantly higher (P<0.001) than that achieved by clopidogrel. The prasugrel peak IPA at 2 h after LD was 93.7% (±6.2%) compared to the clopidogrel peak IPA at 6 h after LD at 65.8% (±17.2%). The VerifyNow and MEA assay yielded results similar to those obtained by LTA.

Conclusions: In healthy Korean subjects, a 30-mg LD of prasugrel yields a more rapid, potent and consistent inhibition of platelet function than a 600-mg LD of clopidogrel. (Circ J 2013; 77: 1253–1259)

Key Words: Clopidogrel; Light transmission aggregometry; Multiple electrode platelet aggregometry; Prasugrel; VerifyNow

D ual anti-platelet therapy (aspirin and clopidogrel) is often recommended for patients undergoing stent implantation with acute coronary syndrome (ACS).1–3

Clopidogrel is a thienopyridine prodrug, which, given orally, is metabolized to an active sulfhydryl form that binds to and irreversibly antagonizes the P2Y12 class of platelet adenosine diphosphate (ADP) receptor.4 When an approved clopidogrel loading dose (LD) regimen of 600 mg is compared to a 300-mg LD, more rapid onset and a higher level of platelet inhibition can be achieved.5,6 While the mechanisms of patient variability and resistance to clopidogrel therapy are not fully understood, genetic, clinical and cellular factors have been investigated.7,8 Several studies have found that clopidogrel resistance has a significant correlation with higher risk of cardiovascular events.9,10

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As with other thienopyridines, prasugrel is an oral antiplatelet agent approved for the reduction of atherothrombotic cardiovascular events in patients presenting with ACS and undergoing percutaneous coronary intervention.11,12 Several studies have reported greater, more rapid and more consistent platelet inhibition with prasugrel than with clopidogrel in healthy subjects as well as in patients with stable coronary artery disease.13–15 Preclinical studies indicate that prasugrel is approximately 10-fold more potent than clopidogrel at inhibiting platelet aggregation, inhibiting thrombus formation and prolonging bleeding times.16 The benefit of prasugrel relative to clopidogrel, however, can be accompanied by a higher risk of major bleeding. The large TRITON-TIMI 38 study provided the first conclusive evidence that prasugrel is associated with excess bleeding relative to clopidogrel (2.4% vs. 1.8%, P=0.03).12 Although the approved LD is 60 mg, previous studies with prasugrel suggest that active metabolite exposure and pharmacodynamic response may be higher in Asian than in Caucasian subjects.17 These findings prompted the current study, in which the pharmacodynamic responses to a lower prasugrel LD were
investigated in Korean subjects.

The aim of this study was to compare the effects of a single prasugrel 30-mg LD to a single clopidogrel LD of 600 mg on the inhibition of platelet aggregation (IPA) in healthy Korean volunteers.

Methods

Subjects and Study Design

The subjects were 12 healthy men, aged 23–28 years (mean, 24.9 years), with body mass index (BMI) of 18.5–28.9 kg/m² (mean, 23.7 kg/m²), screened for a baseline maximum platelet aggregation (MPA) response ≥55% to 10 μmol/L ADP and having standard results on usual clinical tests (including a hepatic function test, renal function test, complete blood count and coagulation study). Clinical laboratory testing was conducted at the Department of Laboratory Medicine, Dong-A University Hospital in Busan, South Korea. The exclusion criteria included the taking of aspirin, other antiplatelet agent or anti-inflammatory drugs, a history of bleeding tendency, reasonable suspicion of vascular malformations, or abnormal coagulation values and the presence of a disorder that might potentially change the absorption, metabolism or removal of the drugs, or those that could constitute a risk to the subject during the study. No concurrent medications were permitted during the study. Each subject provided fully informed consent prior to entering the study.

This was a randomized, 2-way cross-over, open-label study in healthy subjects conducted at a single center from August to September 2011. Twelve subjects were randomly assigned to receive either 30 mg of prasugrel (prasugrel group) or 600 mg of clopidogrel (clopidogrel group) during the initial dosing period. Following a 2-week washout period, the subjects received the alternate thienopyridine (prasugrel or clopidogrel). Each dose of prasugrel or clopidogrel was given with 200 mL of water following an overnight fast. Venous blood samples were obtained for pharmacodynamic measurements before dosing (baseline) and at regular intervals in the 24 h afterwards.

Laboratory Measurements

Blood samples for pharmacodynamic assessment were collected via direct venipuncture at baseline and 0.5, 2, 6, and 24 h after dosage. Platelet aggregation was assessed using 4 methods: traditional light transmission aggregometry (LTA), the VerifyNow P2Y12 assay (Accumetrics, San Diego, CA, USA), multiple electrode platelet aggregometry (MEA, Dynabyte Medical, Munich, Germany).

Platelet Function Test

LTA was performed as previously described. Citrate anticoagulated whole blood was centrifuged at 120 g for 10 min at room temperature to obtain platelet-rich plasma. Platelet-poor plasma was obtained from the remaining specimen by recentrifugation at 1,200 g for 10 min. Platelet-rich plasma was adjusted to a platelet count of 2.5 × 10⁵/μL by adding platelet-poor plasma. Light transmission was calibrated using a cuvette with platelet-poor plasma, equated to 0% and a second cuvette containing platelet-rich plasma equated to 100%. To measure platelet function, 10 μmol/L ADP was added to induce platelet aggregation, before response curves were continuously recorded for 6 min.

The VerifyNow assay (Accumetrics) is a whole-blood, cartridge-based, optical detection system designed to measure platelet aggregation. The ADP P2Y12 receptor is measured in a cartridge channel of the VerifyNow P2Y12 assay containing ADP, a platelet agonist and prostaglandin E₁ (PGE₁), a suppressor of intracellular free calcium level that reduces the non-specific contribution of ADP binding to P2Y1 receptors. The numerical results are then expressed as P2Y12 reaction units (PRU), baseline platelet function independent of P2Y1 receptor inhibition (BASE) and % inhibition. The % inhibition was calculated as follows: ([BASE – PRU]/BASE) × 100%. To avoid confusion of terminology, we define this value as P2Y12 % inhibition, in contrast to % inhibition of PRU.

MEA was performed with a Multiplate Analyzer (Dynabyte Medical). The method has been described in detail elsewhere. Specifically, the adhesion and aggregation of platelets on sensor surfaces enhances the electrical resistance between 2 sensor electrodes. We used an ADP test (6.4 μmol/L ADP) to monitor the antiplatelet effects of clopidogrel. In the test cuvette, hirudin-anticoagulated whole blood (300 μL) was diluted (1:2 vol/vol) with 0.9% NaCl solution for 6.4 μmol/L, and ADP was stirred in for 3 min at 37°C. ADP in the absence of PGE₁ was added, and the increase in electrical impedance was recorded continuously for 6 min and converted into arbitrary aggregation units (AU; 8 AU corresponds approximately to 1 Ohm). The means of the 2 independent determinations were expressed as the area under the curve of aggregation tracing (AUC) in AU·min. The manufacturer recommended the use of arbitrary units (U) to simplify the expression of results (1 U = 10 AU·min = 1 AUC).

Definition of IPA and % Inhibition

Using the LTA, VerifyNow and MEA devices, we recorded MPA, P2Y12 PRU and U.

IPA (% inhibition of MPA) from baseline was calculated using the following formula: % inhibition of MPA = (MPA₀ – MPAₜ)/MPA₀ × 100%, in which MPA₀ is MPA at time (t) after dosing and MPAₜ is MPA at baseline. The decrease in MPA (ΔMPA) was calculated as (MPA₀ – MPAₜ).

Percent inhibition of PRU was determined using the VerifyNow assay. Percent inhibition was calculated using baseline PRU as follows: % inhibition of PRU = (PRU₀ – PRUₜ)/PRU₀ × 100%, where PRU₀ is the baseline PRU before dosing of
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For % inhibition of U, the MEA assays were similar to LTA and VerifyNow.

Comparison of the percentage of pharmacodynamic non-responders in the prasugrel and clopidogrel groups was performed 24h after the LD. For this analysis, a pharmacodynamic non-responder was defined as $10\mu$mol/L ADP MPA $\leq55\%$, $21$ VerifyNow P2Y12 $>240$PRU$^9$ and MEA assay $>46.8$U$^9$ at 24h after the LD.

Safety
Safety assessments performed during volunteer screening visits included physical examination, measurement of vital signs, 12-lead electrocardiogram, hematology, clinical chemistry, coagulation, urinalysis, fecal occult blood test, fundoscopy analysis and examination for petechiae. Adverse events were recorded and monitored throughout the study.
### Results

Twelve healthy male subjects with a mean BMI of 23.7±3.3 kg/m² were enrolled in the study. Both LD treatments were well tolerated, with no volunteer leaving the study due to adverse events. There were no clinically significant bleeding events or occurrences of thrombocytopenia. These data were not statistically different, indicating that the 2-week washout period provided enough time for the reversal of platelet inhibition associated with the first dosing period.

### IPA and % Inhibition

The mean IPA to 10 μmol/L ADP was significantly higher after the prasugrel 30-mg dose than after the clopidogrel 600-mg dose, at all time points (P<0.001; Figure 2A; Table). At 30 min after LD, prasugrel 30 mg achieved a greater mean IPA to 10 μmol/L ADP (70.3%) than clopidogrel 600 mg (21%; P<0.001; Figure 2A). The prasugrel 30-mg LD also achieved greater IPA at 2h (93.7%) and 6h (93.3%) after dosage, compared to the 2-h and 6-h peak IPA for clopidogrel 600 mg (65.7% and 65.8%, all P<0.001). Using the criteria for 10 μmol/L ADP in the LTA assay, 8.3% of subjects at 24 h were classified as pharmacodynamics non-responders to clopidogrel, whereas all subjects responded to the prasugrel 30-mg LD.

As measured by the VerifyNow assay, the mean % inhibition of PRU after a 30-mg dose of prasugrel was significantly higher than for the clopidogrel 600-mg dose at all time points (P<0.001; Figure 2B; Table). Thirty minutes after dosage, the % inhibition of PRU was nearly 6-fold higher in the prasugrel group compared to the 6-h (46.3%) and 24-h (48.5%) peak % inhibition in the clopidogrel group (P<0.001; Figure 2B). Similar to LTA and VerifyNow, the MEA assay showed the mean % inhibition of AUC after a 30-mg dose of prasugrel to be significantly higher than that following a 600-mg dose of clopidogrel at all time points (all P<0.01; Figure 2C; Table). The MEA assay showed that peak % inhibition of PRU was achieved at 2h (91.3%) after dosage in the prasugrel group compared to the 6-h (46.3%) and 24-h (48.5%) peak % inhibition in the clopidogrel group (P<0.001; Figure 2C).

### Comparison of Degree of Inhibition of Platelet Function and Correlation Between Devices

The calculated IPA by LTA testing and % inhibition by VerifyNow assays (IPA and % inhibition of PRU) showed that the 30-mg LD regimen of prasugrel resulted in greater inhibition of platelet function than that measured by MEA (% inhibition of U). The LTA assay after a 600-mg clopidogrel LD, however, showed higher % inhibition than that determined by VerifyNow and MEA (Table). The correlation between the calculation of IPA and % inhibition after LDs of prasugrel and clopidogrel were as follows (baseline up to 24 h after LD): LTA vs. MEA, r=0.73; LTA vs. VerifyNow, r=0.83; and MEA vs. VerifyNow, r=0.67 (P<0.001). As measured by IPA and % inhibition at 6h after dosage response to ADP, prasugrel produced higher and more consistent IPA than clopidogrel (Figure 3).

The VerifyNow device can calculate % inhibition using its...
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In that study, peak levels of prasugrel active metabolites (C_max) were 67% higher in Chinese and 39% higher after adjustment for differences in bodyweight, than for Caucasian volunteers. Taking into account ethnic differences in bodyweight and BMI, the Asian ethnic group could have at least 30–70% higher active metabolite concentrations than the Caucasian group. The present study showed that 10 μmol/L ADP induced 85.9% IPA after 24 h. These results reflect previously published work reporting a 60-mg LD in mean IPA (using 20 μmol/L ADP as agonist) to be 72% for Caucasian, 83% for Chinese, 81% for Korean and 78% for Japanese sub-

Discussion

In the present study, a 30-mg LD of prasugrel achieved faster onset, higher levels, and less variability in the inhibition of platelet activity than an LD of clopidogrel at 600 mg. A faster onset for IPA after prasugrel was evident in a number of parameters. As early as 30 min after LD, the IPA (% inhibition of platelet function) was greater in response to prasugrel than clopidogrel. The maximum effect began to plateau at 2 h after prasugrel compared to 6 h after clopidogrel. The magnitude of IPA or % inhibition was higher at 30 min after LD in the prasugrel group than it was at any point during the 24-h period of observation for the clopidogrel group. In addition, on the basis of previously defined criteria, 8.3–25% of the subjects at 24 h were pharmacodynamics non-responders to the clopidogrel LD, whereas all subjects were classified as responders to the prasugrel LD.

Rationale for Low LD and Maintenance Doses in Asian Patients

In the TRITON-TIMI 38 trial, patients who received prasugrel had improved cardiovascular outcomes but also experienced more bleeding events, compared to those receiving clopidogrel. An ideal dosing regimen would include LD and maintenance doses that are adequate to yield the clinical benefits of increased platelet inhibition without an increased risk of bleeding. Small et al reported that even after normalization for bodyweight, prasugrel active metabolite exposure was approximately 30% higher in Asian subjects when compared with Caucasian subjects after an LD of 60 mg prasugrel, concluding that prasugrel may not be appropriate for Asian subjects. Another study demonstrated that LDs of 60 mg or 40 mg of prasugrel did not show any difference in IPA with 20 μmol/L of ADP. Other reports indicated that 30 mg of prasugrel produces greater platelet inhibition and higher prasugrel active metabolite concentrations than 300 mg of clopidogrel in Chinese subjects.

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Figure 3. Relationship between inhibition of platelet aggregation and % inhibition by 30 mg prasugrel or 600 mg clopidogrel in response to light transmission aggregometry, VerifyNow and multiple electrode aggregometry (MEA), 6 h after loading dose. Subjects were given both prasugrel and clopidogrel in a cross-over fashion.

Figure 4. Relationship between reported VerifyNow % inhibition and calculated % inhibition of P2Y12 reaction units from baseline before medication. Correlation coefficient (r) was calculated by the Pearson method. Blue circles, clopidogrel; red triangles, prasugrel. Symbols represent individual simultaneous measurements at baseline up to 24 h after loading dose.
jects at 24h afterwards.\textsuperscript{17} The VerifyNow % inhibition of PRU for the prasugrel group and the clopidogrel group at 2h was 91.3\% vs. 43.1\%, respectively. These results are also in line with those reported for Swedish subjects using a 60-mg LD of prasugrel and a 600-mg LD of clopidogrel at 2h, as 93.8\% vs. 43.9\%.\textsuperscript{26} The present results suggest that a lower LD of prasugrel (30mg) might be an alternative option for patients of East Asian ethnicity. These findings need to be validated in coronary stent patients in the future. Regarding the pharmacodynamics of prasugrel maintenance doses in Caucasian subjects, Jakubowski et al tested multiple doses of prasugrel (20mg, 10mg, 5mg), which resulted in a dose-dependent inhibition of platelet activity and IPA (5×10$^{-9}$/L, ADP) that were significantly greater with prasugrel (77.2\%, 70.5\% and 52.7\%) compared with clopidogrel 75mg (36.5\%).\textsuperscript{14} In an Asian study, Yokoi et al compared several different LDs and MDs (10-mg LD/2.5-mg MD, 15-mg LD/3.75-mg MD, 20-mg LD/5-mg MD and 300-mg clopidogrel LD and 75-mg MD).\textsuperscript{26} In that study, a 10-mg LD/2.5-mg MD regimen was similar to clopidogrel 300-mg LD/75-mg MD. These dosages are 1/6 and 1/4 of the current manufacturer recommended LD and MD.

**Differences in Metabolic Pathways Between Prasugrel and Clopidogrel**

In relation to clopidogrel, the faster onset of prasugrel platelet aggregation effects can be attributed to a difference in the metabolic pathways leading to the formation of the respective active metabolites.\textsuperscript{23,27,28} Prasugrel and clopidogrel require cytochrome P450 (CYP) metabolism for the generation of active metabolites, but the pathways leading to these metabolites differ between the pro-drugs.\textsuperscript{23} The esterase-mediated step for prasugrel occurs predominantly in the intestine, as does the CYP-mediated oxidative step leading to active metabolite formation. Hydrolysis of the remaining 15\% of clopidogrel, however, also occurs by 2 CYP-dependent steps. The slower metabolic alteration of clopidogrel lowers the rate and level of active metabolite formation compared to the process for prasugrel active metabolite formation.\textsuperscript{29} Differences in the metabolism of the 2 drugs also lead to markedly different plasma concentrations of the active metabolites. The exposure to prasugrel following an LD of 60mg was approximately 12-fold greater than that following 300-mg clopidogrel.\textsuperscript{30} The metabolism of prasugrel to its active metabolite therefore has greater pharmacokinetic benefits than the same process for clopidogrel. These differences underpin the relative efficacy of prasugrel vs. clopidogrel.

**Assessment of IPA or % Inhibition After Prasugrel and Clopidogrel LD**

The pharmacodynamic responses to prasugrel, as measured by IPA and % inhibition, were also consistently higher than responses to clopidogrel. Given the present cross-over study design, individual responses to each agent and the degree of variability of IPA and % inhibition could be assessed. It was observed from the intra-individual responses to the 2 agents that although variability was present in response to both agents, each individual participant had a significant increase in IPA (as measured through the different tests) with prasugrel relative to clopidogrel (Figure 3). In addition, even in subjects with poor response to clopidogrel, platelet inhibition was successful following prasugrel, thereby minimizing the possibility of variations in P2Y12 function potentially causing clopidogrel response variability.

IPA and % inhibition of PRU was >90\% on LTA and the VerifyNow after prasugrel, but on MEA it was <80\% platelet inhibition using the present standard definitions. The LTA and MEA assays use ADP as an agonist for assessing P2Y12 effects, which can confound the results through the possible activation of ADP receptor subtypes other than P2Y12, contributing to platelet aggregation. In contrast, VerifyNow uses 2 reagents (ADP and PGE1) as contributors to ADP-induced aggregation via the P2Y1 receptor in response to ADP. Therefore, it can be concluded that VerifyNow is more suitable for comparing the specific P2Y12 inhibitory effects of thienopyridine. This may also partially explain inconsistencies in data arising from the different devices.

**Study Limitations**

This study was performed with normal male volunteers without aspirin medication, which is not always reflective of typical patients. We did not measure the active metabolites of prasugrel and clopidogrel.

**Conclusions**

In this direct cross-over comparison of healthy Korean subjects, a 30-mg LD of prasugrel yielded a more rapid, potent and consistent inhibition of platelet function than a 600-mg LD of clopidogrel.

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**Disclosures**

The authors declare no conflicts of interest.

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