Prasugrel achieves greater and faster P2Y\textsubscript{12} receptor-mediated platelet inhibition than clopidogrel due to more efficient generation of its active metabolite in aspirin-treated patients with coronary artery disease

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Aims

P2Y\textsubscript{12} receptor antagonism and platelet inhibition by prasugrel vs. clopidogrel were investigated in patients with stable coronary artery disease.

Methods and results

One hundred and ten aspirin treated subjects were randomized to double-blind treatment with clopidogrel (n = 55) 600 mg loading dose (LD) and 75 mg maintenance dose (MD) or prasugrel (n = 55) 60 mg LD and 10 mg MD for 28 days. Concentrations of prasugrel and clopidogrel active metabolites were determined. Platelet aggregation to 20 \textmu M adenosine diphosphate, measured by light transmission aggregometry, was reported as maximal platelet aggregation (MPA). P2Y\textsubscript{12} function was assessed by the vasodilator-stimulated phosphoprotein assay and reported as platelet reactivity index (PRI). The same pharmacodynamic measurements were performed after ex vivo addition of clopidogrel’s active metabolite. At 2 h post-LD, mean MPA was 31 vs. 55%, and mean PRI 8.3 vs. 55.9% for prasugrel and clopidogrel, respectively (P < 0.001). During MD on day 14 and 28, mean MPA was 42 vs. 54% and mean PRI was 25 vs. 51%, respectively (P < 0.001). Peak level of the active metabolite and P2Y\textsubscript{12} inhibition occurred earlier and was greater with prasugrel (P < 0.001). Mean area under the time-concentration curve (AUC; \textmu M.h) of the respective active metabolite was higher with prasugrel vs. clopidogrel post-LD (1.11 vs. 0.24) and post-MD (0.16 vs. 0.062). Ex vivo addition of clopidogrel’s active metabolite further reduced PRI in all patients whose platelets were not already maximally inhibited.

Conclusion

In aspirin-treated subjects with coronary artery disease, prasugrel 60/10 mg provides faster onset and greater inhibition of P2Y\textsubscript{12} receptor-mediated platelet aggregation than clopidogrel 600/75 mg, because of greater and more efficient generation of the active metabolite.

Keywords

Trials • Platelets • Coronary artery disease • Clopidogrel • Prasugrel
Background

Dual antiplatelet therapy with aspirin and clopidogrel is recommended for patients with acute coronary syndrome (ACS) and patients undergoing stent implantation. The currently approved clopidogrel dosing regimen consists of a 300 mg loading dose (LD) followed by a maintenance dose (MD) of 75 mg co-administered with aspirin. A more rapid onset and higher level of platelet inhibition can be obtained with a clopidogrel 600 mg LD, a dose recently endorsed for ACS to obtain better protection during percutaneous coronary interventions. Several studies have shown poor responsiveness to clopidogrel in 5–44% of treated patients. In these patients, there seems also to be a higher risk of cardiovascular events. The mechanisms for the poor response to clopidogrel in some patients are unclear although genetic, metabolic, cellular, and clinical factors have been proposed.

Like clopidogrel, the novel thienopyridine adenosine diphosphate (ADP) receptor antagonist, prasugrel (CS-747, LY640315) is an orally administered prodrug that, after absorption, is converted to an active metabolite that inhibits platelet aggregation via antagonism of the P2Y12 receptor. Compared to standard doses of clopidogrel, prasugrel is more efficiently metabolized to its active metabolite providing a more pronounced platelet inhibition with less inter-subject variability.

The aims of this study were to compare the pharmacodynamic and pharmacokinetic effects of a prasugrel 60 mg LD and 10 mg once daily MD regimen with clopidogrel using the higher 600 mg LD and standard 75 mg once daily MD, in aspirin-treated patients with coronary artery disease. To further elucidate the mechanism of variability in thienopyridine response, we assessed pharmacokinetics of the active metabolites of clopidogrel and prasugrel, and measured the antiplatelet effects after ex vivo addition of clopidogrel’s active metabolite.

Methods

Design

This randomized, double-blind, double-dummy, two-arm parallel-group study was conducted in adult male and female patients with stable coronary artery disease, aged 40 to 75 years (Table 1). Two centres in Sweden enrolled patients from April 2006 to December 2006. Ethical review board approval was obtained and the study was conducted according to the ethical principles of Declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided signed informed consent.

Subjects were enrolled in the study if they had stable coronary artery disease defined as chronic stable angina, prior history of unstable angina or myocardial infarction, previous coronary revascularization or disease of at least one coronary vessel on previous angiography or non-invasive imaging procedure.

Subjects were excluded for: unstable coronary artery disease within the previous 30 days; coronary artery intervention within the previous 90 days, or planned within 40 days following randomization; history or presence of bleeding disorder; and history of recent surgery or severe trauma, uncontrolled hypertension, arrhythmia, or severe congestive heart failure. Intake of thienopyridines ≤10 days prior to screening, other antiplatelet or anticoagulant agents besides aspirin within 30 days of screening was not allowed. Subjects receiving treatment with non-steroidal anti-inflammatory drugs or cyclo-oxygenase-2 inhibitors that could not be discontinued for the duration of the study were also excluded.

Subjects were assigned to either clopidogrel or prasugrel through an interactive voice response system at a randomization ratio of 1:1 at site level using random permuted blocks of size 4 (block size blinded until after data lock). To preserve blinding throughout the study, a minimum number of sponsor personnel not involved in the conduct of the study or analysis of the results had access to randomization tables and treatment assignments until study completion. No unblinding was performed throughout the study.

Subjects visited the research units in Uppsala or Lund on five different occasions. An initial screening examination was performed followed by the lead-in period on aspirin. During the study, subjects visited the research unit at the following occasions: Day 1, Day 2, Day 14 ± 3 days, and a final visit Day 29 ± 3 days. Blood samples for bioanalytical and pharmacodynamic assays were taken by direct puncture of an antecubital vein at predefined time points.

| Table 1 Baseline characteristics of all enrolled subjects |
|-----------------------------------------------|--------------------------|
|                                              | Prasugrel               | Clopidogrel |
|                                              | 60 mg LD/10 mg MD,     | 600 mg LD/75 mg MD, |
|                                              | N = 55 (%)              | N = 55 (%)    |
| Gender                                       |                         |             |
| Male                                         | 48 (87)                 | 53 (96)     |
| Female                                       | 7 (13)                  | 2 (4)       |
| Age (years)                                  | 62 ± 6.1                | 64 ± 6.2    |
| Mean (SD)                                    | 47–73                   | 47–75       |
| Range                                        |                         |             |
| Body weight (kg)                             | 87.3 ± 13.5             | 84.3 ± 11.7 |
| Mean (SD)                                    | 51.5–143.6              | 65.3–125.0  |
| Range                                        |                         |             |
| Hypertension                                 | 38 (69)                 | 33 (60)     |
| Diabetes mellitus                            | 11 (20)                 | 9 (16)      |
| Hyperlipidaemia                              | 47 (85)                 | 52 (95)     |
| Stable angina pectoris                       | 31 (56)                 | 33 (60)     |
| Prior unstable angina                        | 10 (18)                 | 2 (4)       |
| Prior myocardial infarction                  | 37 (67)                 | 39 (71)     |
| Congestive heart failure                     | 4 (7)                   | 9 (16)      |
| Prior stroke                                 | 0 (0)                   | 1 (2)       |
| Prior coronary artery bypass graft           | 2 (4)                   | 7 (13)      |
| Prior percutaneous coronary intervention      | 54 (98)                 | 54 (98)     |
| Angiography showed ≥50% stenosis of major epicardial vessel | 54 (98) | 55 (100) |
| ACE-inhibitor or ARB                         | 35 (64)                 | 40 (73)     |
| Beta-blocker                                 | 44 (80)                 | 46 (84)     |
| Statin                                       | 48 (87)                 | 52 (95)     |
samples for the measurement of platelet aggregation were collected in 3.8% sodium citrate. Laboratory safety tests were collected and monitored throughout the study.

The sample size was determined by assuming mean changes from baseline in maximal platelet aggregation (MPA) to 20 μM ADP at 2 h post-LD of 44.2% for 60 mg LD of prasugrel (observed change) and 32.4% for 600 mg LD of clopidogrel (twice the observed change to 300 mg LD of clopidogrel) with a standard deviation (SD) of 15%, based on a previously performed study on patients with stable atherosclerosis. This phase Ib study was not registered in a clinical trials registry.

**Patients**

Eligible patients were identified in the Swedish Coronary and Angioplasty Registry or the Registry on Cardiac Intensive Care. Of 114 screened patients, 112 satisfied inclusion criteria: 110 subjects were enrolled in the study (101 males, 9 females). Of four subject discontinuations, two were due to subject decision (randomized to clopidogrel, n = 2) and two at the request of the investigator due to inadequate venous access (randomized to clopidogrel, n = 1) and pre-existing anaemia (randomized to prasugrel, n = 1). Thus, a total of 106 subjects completed the study, according to protocol, 54 on prasugrel, and 52 on clopidogrel. There was no significant difference in baseline clinical characteristics except for a small difference in age (< 3 years) (Table 1).

**Medication**

All study subjects received aspirin 75 mg once daily for a run in period of 5-21 days prior to randomization and continued throughout the study. Aspirin was not considered as an investigational drug but was provided (1 bottle, 100 tablets) in the study ensuring that all subjects received the same daily dose. Following the open-label aspirin run-in period, subjects continuing to meet enrolment criteria were assigned to blinded treatment with either clopidogrel or prasugrel. All patients were administered a LD of either prasugrel 60 mg or clopidogrel 600 mg on Day 1 followed by either prasugrel 10 mg or clopidogrel 75 mg as a once daily MD for a total of 28 ± 3 days. On Day 1, 2, 14 ± 3 and 29 ± 3 study medications were administered at the study site after an overnight fast.

**Pharmacodynamic analyses**

**Light transmission aggregometry (LTA)**

ADP-induced platelet aggregation was measured in platelet-rich plasma by light transmission aggregometry (LTA) on Day 1 at baseline (predose), 30 min and 1, 2, 4 h post-LD, and pre-dose during the MD period on Day 2 (24 h ± 4 h post-LD), Day 14 ± 3 and Day 29 ± 3. LTA was performed within 180 min from venipuncture on a BioData PAP-4 optical aggregometer, with temperature maintained at 37°C and using each subject's platelet-poor plasma to set 100% light transmission. Platelet aggregation was allowed to proceed for approximately 7 min following addition of 5 μM or 20 μM ADP. MPA was recorded as the highest value achieved during this observation period. In addition, the mean change from baseline (pre LD) in MPA (ΔMPA) was calculated for each time point and used for some statistical analyses.

**vasodilator-stimulated phosphoprotein**

The vasodilator-stimulated phosphoprotein (VASP) assay was performed using a commercially available method according to the manufacturer's specifications (Biocytex Platelet VASP kit, Marseille, France). Samples from Uppsala were analysed on an Epics XL from Beckman Coulter, and samples from Lund were analysed on a FACSV

Scan from Becton Dickinson. Synchronization between the flow cytometers was performed. The platelet reactivity index (PRI %) was calculated from the corrected mean fluorescence intensity (cMFI) following incubation of the platelets with either prostaglandin E1 alone or prostaglandin E1+ADP as follows:

\[
PRI\% = \frac{cMFI_{PGE1}}{cMFI_{PGE1+ADP}} \times 100\%
\]

The VASP assay was performed on samples obtained prior to the LD and at 1, 2, and 24 h after the LD and prior to the daily MD at Day 14 ± 3 and Day 29 ± 3.

**Pharmacokinetic analyses**

**Concentration of active metabolite**

Plasma concentrations of the prasugrel active metabolite (R-138727) and clopidogrel active metabolite (R-130964) were analysed in samples obtained at 30 min, 1 h, 2 h, 4 h, and 6 h post-LD and during the MD period on Day 2, Day 14, and Day 29 at 30 min, 1, 2, and 4 h post-MD. A 25 μM volume of 2-bromo-3'-methoxycacetophenone (500 mM in acetone) was added to a 4 mL sample of blood collected in EDTA within 30 s of collection in order to stabilize the active metabolite. Active metabolites were assayed using validated liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) methods at Advion Biosciences, Inc., Ithaca, NY, USA with a lower limit of quantification of 0.5 ng/mL. Samples of the active metabolite of prasugrel and clopidogrel collected within ± 10% of the scheduled post-dose sampling times were included in the presentation of the mean concentration-time plot. The area under the curve (AUC) of prasugrel’s and clopidogrel’s respective active metabolites in individual patients was estimated using an established population pharmacokinetic model.

**Addition of clopidogrel active metabolite ex vivo**

Ten micromolar (final concentration) of clopidogrel active metabolite was added ex vivo to samples collected at baseline (pre LD) and on Day 29. The samples were incubated for 30 min at 37°C and then P2Y12 receptor inhibition was characterized using both VASP (PRI) and LTA (MPA to 5 and 20 μM ADP). The final concentration of clopidogrel active metabolite was chosen following preliminary titration experiments indicating that this concentration was the minimum concentration that maximally inhibited platelet function. In these experiments, addition of vehicle control did not affect the measurements of platelet function. The clopidogrel active metabolite was provided by Sankyo Co., Ltd, Tokyo, Japan.

**Adverse events**

The safety of the study participants was monitored throughout the study with clinical assessment at specific occasions. Unexpected signs and symptoms were recorded throughout the treatment period.

**Statistical analyses**

The primary outcome, MPA to 20 μM ADP at 2 h post-LD, was compared between LDs using analyses of covariance (ANCOVA) with treatment and study site as fixed factors and baseline MPA as a covariate. ANCOVA analyses with the 2 h post-LD result as the response were also performed for MPA to 20 and 5 μM ADP, ΔMPA to 5 μM ADP, IPA to 20 and 5 μM ADP, PRI, and change in PRI with treatment and study site as fixed factors. For ANCOVA analyses using MPA, ΔMPA, PRI, or ΔPRI, the baseline value was used as a covariate. For the MPA LD/MD analyses, a linear mixed effect model
was employed with MPA to 20 μM ADP at each time as responses, with treatment, time, the treatment by time interaction and study site as fixed effects, subject as a random effect, and baseline MPA as a covariate. The unstructured covariance matrix was assumed and allowed to differ for the two treatments. Similar linear mixed effect models were employed for MPA to 5 μM ADP and for PRI. For each model, the differences between the treatments at each time point were calculated with 90% CI of the differences and P-values. Data from the clopidogrel active metabolite spiking experiment (MPA and PRI) were analysed to compare the ex vivo active metabolite-treated results to untreated samples from the same individual at time points relative to LD and MD.

The geometric mean AUC for the active metabolite, with corresponding 90% confidence limits, were calculated for the prasugrel and clopidogrel groups, respectively, at time points relative to LD and MD. The relationship between the logarithmically transformed AUC and PRI was evaluated with Pearson correlation. The software used for statistical analyses was SAS Version 8.2, SAS Institute Inc., Cary, NC, USA. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Platelet aggregation evaluated by light transmission aggregometry

There was no significant difference in MPA levels at baseline. Mean MPA values were significantly (P < 0.001) lower with prasugrel in the first sample 30 min post-LD and at all time-points thereafter (Figure 1). At 2 h, the mean MPA was significantly lower with prasugrel (31%) compared with clopidogrel (54.7%; P < 0.001). The ΔMPA at 2 h post-LD was −41.6 for prasugrel and −17.7 for clopidogrel (P < 0.001). On maintenance treatment (Days 14 and 29), the MPA was still significantly lower with prasugrel (Figure 1) (Table 2). The mean MPA for prasugrel and clopidogrel was 34.3 vs. 54.8% at 24 h post-LD and 42.6 vs. 53.5% on Day 29 (P < 0.001). The MPA to 5 μM ADP showed similar differences between dosing regimens to those seen with 20 μM ADP (data not shown).

Inhibition of P2Y12 receptors evaluated by the vasodilator-stimulated phosphoprotein assay

P2Y12 receptor blockade, as indicated by a lower PRI using the VASP assay, was significantly greater at 1, 2, and 24 h following the prasugrel LD (PRI of 16.0, 8.3, and 8.7%) compared with the clopidogrel LD (PRI of 68.5, 55.9, and 50.2%; P < 0.001), (Figure 2, Table 3). During maintenance treatment, the mean PRI on prasugrel treatment remained approximately 50% below that on clopidogrel (P < 0.001). During maintenance treatment with prasugrel, the mean PRI was higher than after the prasugrel LD (24.7% and 25.0% on Days 14 and 29, respectively). In contrast, the mean PRI during maintenance treatment with clopidogrel at the same time points, 50.7 and 51.2% respectively, was similar to that seen after the clopidogrel LD, but still higher than prasugrel MD values.

Pharmacokinetics of active metabolite following oral administration of study drug

Following administration of a prasugrel LD, peak concentrations of active metabolite occurred earlier, and were higher than those following administration of a clopidogrel LD (Figure 3A). Similarly, the prasugrel active metabolite peak concentrations after daily MDs were consistently higher than those following clopidogrel MDs. Results from Day 14 (data not shown) were similar to those from Day 29 (Figure 3B). AUC (μM-h) was higher with prasugrel vs. clopidogrel both after the LD, 1.11 (90% CI 1.02–1.19) vs. 0.24 (90% CI 0.22–0.26) and the MD, 0.16 (90% CI 0.15–0.17) vs. 0.062 (90% CI 0.057–0.067).

Relationship between active metabolite exposure and platelet reactivity index (vasodilator-stimulated phosphoprotein assay)

Compared to a clopidogrel 600 mg LD, the higher exposure to active metabolite after a prasugrel 60 mg LD produced a lower PRI with smaller inter-subject variability. An AUC of active metabolite above ~0.8 μM-h did not further reduce PRI (Figure 3C) indicating that the 60 mg prasugrel LD produced a maximal effect on P2Y12-mediated platelet inhibition. Conversely, the lower exposure to active metabolite after the clopidogrel LD produced sub-maximal inhibition of P2Y12-mediated platelet aggregation, which was associated with a higher and more variable PRI (Figure 3C). During MD, the relationship between log AUC of active metabolite and PRI was similar for prasugrel and clopidogrel (r = −0.81, P < 0.001). In the region with a higher AUC of active metabolite during clopidogrel MD, there was an overlap.
with active metabolite after prasugrel MD and a corresponding overlap in effect on PRI [Figure 3D]. The results were similar for MPA (data not shown).

**Active metabolite spiking**

The ex vivo addition of clopidogrel’s active metabolite to blood samples taken pre-LD or pre-MD resulted in drops in MPA and PRI to low levels in all samples. In clopidogrel-treated patients, the addition of clopidogrel’s active metabolite ex vivo reduced MPA and PRI to low levels with small inter-individual variability regardless of the ex vivo response to the 600 mg LD or 75 mg MD of clopidogrel (Figures 4A and 5A). In prasugrel-treated patients, the MPA and PRI levels following addition of clopidogrel’s active metabolite were similar to the samples 2 h after a prasugrel 60 mg LD, which were already at maximum effect but lower than those in samples post-MD in patients not maintaining a maximal effect during MD (Figures 4B and 5B).

**Adverse events**

Oral doses of prasugrel 60 mg LD/10 mg MD and clopidogrel 600 mg LD/75 mg MD were both well tolerated on a background of aspirin 75 mg once daily. The incidence of total adverse events was similar for both treatment regimens. However, the incidence of bleeding-related events, predominately venipuncture-related minor bleeds and bruising, was higher for the prasugrel dosing regimen (overall 32 bleeding and bruising adverse events, all causalities) compared to 13 in the clopidogrel group. No subjects discontinued from the study due to an adverse event.

**Discussion**

In accordance with previous studies in healthy volunteers and in patients with stable atherosclerosis, we showed that prasugrel achieves faster and greater inhibition of ADP-induced platelet aggregation, even when compared to clopidogrel starting with a 600 mg LD. Importantly, the mean difference in the inhibition of platelet aggregation between clopidogrel and prasugrel was observed as soon as 0.5 h after the LD, at which time prasugrel had provided the magnitude of effect not seen with clopidogrel until after 2–4 h. This difference in speed of onset and magnitude of the platelet inhibitory effect might be clinically important in the treatment of ACS patients and during percutaneous coronary interventions.

The MPA to 20 μM ADP during maintenance doses with prasugrel 10 mg remained significantly lower than with clopidogrel 75 mg, although the difference was halved in comparison to after LD. Recently, a clopidogrel MD of 150 mg has been associated with greater inhibition of platelet aggregation than the 75 mg standard dose. However, the present MD of both prasugrel and clopidogrel have been selected for comparison in the ongoing TRITON-TIMI 38 pivotal trial, based on estimates of the optimal balance between efficacy and safety from earlier phase II dose-ranging trials.

LTA using ADP as the agonist for assessing P2Y12 effects is confounded as ADP receptor subtypes other than P2Y12 can be activated and contribute to platelet aggregation. The assay system for phosphorylation of VASP uses two reagents, ADP and PGE1. The addition of ADP to PGE1 stimulated platelets does not

### Table 2 Summary of maximal platelet aggregation (20 μM ADP agonist) after prasugrel or clopidogrel loading and maintenance doses

<table>
<thead>
<tr>
<th>Day</th>
<th>Time (h)</th>
<th>Prasugrel, LS mean (90% CI), 60 mg LD/10 mg MD</th>
<th>Clopidogrel, LS mean (90% CI), 600 mg LD/75 mg MD</th>
<th>Difference LS mean (90% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Predosea</td>
<td>72.9 (71.8, 74.0)</td>
<td>72.6 (71.5, 73.7)</td>
<td>-27.3 (-30.8, -23.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1a</td>
<td>37.8 (34.8, 40.7)</td>
<td>65.1 (63.1, 67.0)</td>
<td>-23.7 (-27.0, -20.5)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>31.0 (29.2, 32.9)</td>
<td>54.7 (52.1, 57.4)</td>
<td>-20.5 (-23.4, -17.3)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>24 ± 4a</td>
<td>34.3 (32.6, 36.0)</td>
<td>54.8 (52.4, 57.2)</td>
<td>-11.9 (-14.7, -9.20)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>14 ± 3</td>
<td>Predoseb</td>
<td>41.6 (39.8, 43.4)</td>
<td>53.5 (51.5, 55.5)</td>
<td>-10.9 (-14.3, -7.53)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>29 ± 3</td>
<td>Predoseb</td>
<td>42.6 (40.3, 44.9)</td>
<td>53.5 (51.0, 56.0)</td>
<td>-10.9 (-14.3, -7.53)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*aLoading dose.

*bMaintenance dose.
Table 3 Summary of vasodilator-stimulated phosphoprotein phosphorylation, platelet reactivity index (PRI, %), after prasugrel or clopidogrel loading and maintenance doses

<table>
<thead>
<tr>
<th>Day</th>
<th>Time (h)</th>
<th>Prasugrel, LS mean (90% CI), 60 mg LD/10 mg MD</th>
<th>Clopidogrel, LS mean (90% CI), 600 mg LD/75 mg MD</th>
<th>Difference, LS mean (90% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Predosea</td>
<td>81.1 (80.0, 82.2)</td>
<td>81.9 (80.8, 83.0)</td>
<td>− 52.5 (− 58.8, − 46.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1a</td>
<td>16.0 (12.3, 19.7)</td>
<td>68.5 (63.5, 73.6)</td>
<td>55.9 (50.8, 61.0)</td>
<td>− 47.6 (− 53.9, − 41.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2</td>
<td>8.32 (4.61, 12.0)</td>
<td>50.2 (45.1, 55.2)</td>
<td>50.2 (45.1, 55.2)</td>
<td>− 41.5 (− 47.8, − 35.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>24 ± 4</td>
<td>8.68 (4.93, 12.4)</td>
<td>26.0 (22.3, 27.7)</td>
<td>58.8 (54.6, 63.0)</td>
<td>51.2 (47.0, 55.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>14 ± 3</td>
<td>Predoseb</td>
<td>24.7 (22.0, 27.4)</td>
<td>50.7 (46.6, 54.8)</td>
<td>26.0 (21.1, 30.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>29 ± 3</td>
<td>Predoseb</td>
<td>25.0 (22.3, 27.7)</td>
<td>51.2 (47.0, 55.3)</td>
<td>26.2 (21.3, 31.2)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

aLoading dose.
bMaintenance dose.

Figure 3 (A) Plasma concentrations of prasugrel and clopidogrel active metabolites following administration of a single loading dose. Lower limit of quantitation was 0.5 ng/mL. Data presented as mean ± SD. Closed circle represents prasugrel 60 mg LD (N = 55). Open circle represents clopidogrel 600 mg LD (N = 54). (B) Plasma concentrations of prasugrel and clopidogrel active metabolites following administration of maintenance doses. Lower limit of quantitation was 0.5 ng/mL. Data presented as mean ± SD. Closed circle represents prasugrel 10 mg MD (N = 54 on Day 29 ± 3). Open circle represents clopidogrel 75 mg MD (N = 54 on Day 29 ± 3). (C) Scatter plot of platelet reactivity index (PRI, %) at 24 h vs. area under the curve (AUC; μM·h) of active metabolite after a loading dose of clopidogrel 600 mg or prasugrel 60 mg. Closed circle represents patients on prasugrel. Open circle represents patients on clopidogrel. (D) Scatter plot of platelet reactivity index (PRI, %) vs. area under the curve (AUC; μM·h) of active metabolite after maintenance dose of clopidogrel 75 mg or prasugrel 10 mg. Closed circle represents patients on prasugrel. Open circle represents patients on clopidogrel.
reduce VASP phosphorylation if P2Y12 receptors are successfully inhibited by a thienopyridine, as this effect of ADP is mediated by P2Y12 but not P2Y1. Therefore, flow cytometric assessment of VASP phosphorylation is more suitable for comparing the specific P2Y12 inhibitory effects of thienopyridines. The VASP results in the present study showed a 90% inhibition of P2Y12 receptors at 1–2 h after a 60 mg LD of prasugrel which decreased to 66% inhibition during the 10 mg MD after 14 and 29 days. In contrast, at no time point did a 600 mg LD of clopidogrel induce more than a 40% inhibition of P2Y12 receptors. Thus, the VASP analyses indicated a larger difference in P2Y12 inhibition between the treatments than the MPA measurements, the latter also reflecting P2Y1 receptor mediated aggregation not inhibited by thienopyridines.

Compared to clopidogrel, the more rapid onset and greater inhibition of P2Y12-mediated platelet aggregation, as measured by MPA, with prasugrel was associated with earlier and higher peak levels and greater exposure to the active metabolite. Furthermore, inhibition of P2Y12-induced platelet reactivity, as measured by VASP, was closely related to the exposure of active metabolites, for prasugrel LD up to near saturation levels of the P2Y12 receptor, as indicated by PRI values approaching zero with no further change with increasing plasma levels. During MD, there was good log-linear correlation between platelet inhibition and exposure to the active metabolite, indicating that the pharmacokinetic/pharmacodynamic relationship between clopidogrel’s and prasugrel’s active metabolites was similar. These data are consistent with

**Figure 4** (A) Individual levels of maximal platelet aggregation (MPA) before and after addition of active metabolite for subjects on clopidogrel. Pre-LD = baseline sample, before start of treatment. Post-LD 2 h = 2 h after loading dose (LD). Act-met Pre-LD = sample before LD, analysed after addition of active metabolite. MD 29 d = Day 29, maintenance treatment. Act Met 29 d = sample before maintenance dose Day 29, analysed after addition of active metabolite. The results in each individual subject are connected by a line. The individual results from three subjects were omitted due to laboratory errors. (B) Individual levels of maximal platelet aggregation (MPA) before and after addition of active metabolite for subjects on prasugrel. Signs and symbols as in Figure 4A. The results in each individual subject are connected by a line. The individual results from three subjects were omitted due to laboratory errors.
those of Sugidachi and Small, and show that the differences in PRI and MPA are caused by differences in active metabolite exposure and not by differences in potencies of the active metabolites.

Addition of the active metabolite to blood samples ex vivo resulted in a further decrease in MPA and PRI for all subjects not already at maximal levels of the P2Y12 receptor inhibition. These findings suggest that all patients’ P2Y12 receptors will respond to thienopyridine inhibition provided that the concentration of the active metabolite reaches an adequate level. The addition of the active metabolite further reduced P2Y12 reactivity in all subjects receiving the high clopidogrel LD, indicating that maximal inhibition will not be reached even after the higher oral LD of clopidogrel. In contrast, there was no further reduction in P2Y12 reactivity after prasugrel LD while a decrease was seen in many patients during prasugrel MD. This pattern reflects maximal and submaximal P2Y12 receptor blockade after a prasugrel LD and during MD, respectively. In a recent study on human and rat platelets, the active metabolites of both prasugrel and clopidogrel showed similar ex vivo antiplatelet activity, thus supporting the hypothesis that prasugrel’s greater antiplatelet effect is related to more efficient generation of the active metabolite when compared with clopidogrel. Therefore, the variable inhibition of platelet aggregation observed with clopidogrel seems to result from lower exposure to the active metabolite, rather than on the presence of P2Y12 receptor heterogeneity.

Study limitations
This study evaluated a small population of patients with stable coronary artery disease for a limited time period in an experimental setting, thus restricting the results to the trial’s pharmacokinetic and pharmacodynamic objectives. Therefore, in this study there is limited information regarding the possible factors that influence the results related to the doses used and/or the extent of P2Y12 blockade achieved by the study drugs if used in other clinical settings. Differences in the effects on platelet activity between this stable situation and the acute coronary setting cannot be excluded although comparable inhibition of platelet aggregation to 20 μM ADP for the 600 mg clopidogrel LD was observed in a recent trial that compared different clopidogrel LD in 103 patients with non-ST-segment-elevation ACS.

Conclusion
In aspirin-treated subjects with coronary artery disease, a prasugrel 60 mg LD and 10 mg MD provided faster onset and more pronounced inhibition of P2Y12 receptor-mediated platelet aggregation than a high (600 mg) LD of clopidogrel and a 75 mg MD. This difference resulted from faster, greater, and more efficient generation of the active metabolite from prasugrel than clopidogrel. As ex vivo addition of the active metabolite led to maximal inhibition of aggregation in all patients, a poor response to clopidogrel is explained by an ineffective generation of its active metabolite, rather than from P2Y12 receptor heterogeneity.

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**CLINICAL VIGNETTE**

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Complete occlusion of the aortic isthmus

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A 44-year-old man was admitted to hospital with severe chest pain. The ECG showed a subacute myocardial infarction. An attempt of interventional revascularization from the femoral artery failed because the catheter could not be advanced via the thoracic aorta. A brachial approach showed a coronary two-vessel disease with occlusion of the circumflex artery. Despite successful revascularization, the left ventricular ejection fraction remained severely depressed (20%). A CT scan revealed complete occlusion of the descending aorta distal to the origin of the left subclavian artery (Panel A). Interventional reconstitution of the aortic continuity was planned to reduce the left ventricular afterload. A pigtail catheter was placed proximal to the occlusion from a left radial access. An additional left femoral approach allowed simultaneous contrast injection showing the interrupted aortic segment (Panel B), which was then perforated with a transseptal needle and a Brockenbrough catheter (Panel C). The transseptal needle was exchanged for an Amplatz superstiff wire. A covered CP stent mounted on a 15 mm Cordis Maxi balloon catheter was implanted through a Cook blue long sheath (Panel D). The post-interventional angiography proved correct stent position without any pressure gradient, dissection, or aneurysm (Panel D). After 4 weeks, an echocardiographic control showed improved left ventricular ejection fraction (35%).

It remains speculation whether this was a primarily interrupted aortic arch (type A) or a secondarily atretic coarctation.

Panel A. CT scan as a reconstruction (lateral view) of the thorax and abdomen, showing the interruption of the descending aorta at the isthmic level.

Panel B. Simultaneous angiogram of the superior and inferior parts of the descending aorta (RAO view), sparing the atretic segment.

Panel C. Perforation of the atretic segment from a femoral access using a standard transseptal needle and a Brockenbrough catheter (RAO view).

Panel D. Postinterventional angiography showing the implanted CP stent (RAO view).

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