Absorption, Metabolization, and Antiplatelet Effects of 300-, 600-, and 900-mg Loading Doses of Clopidogrel

Results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) Trial

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Background—For patients undergoing percutaneous coronary intervention, the administration of a clopidogrel loading dose ranging from 300 to 600 mg is currently recommended. It is unknown, though, whether loading doses higher than 600 mg exert additional suppression of platelet function.

Methods and Results—Sixty patients with suspected or documented coronary artery disease admitted to our hospital for coronary angiography were included in this trial. They were allocated to 1 of 3 clopidogrel loading doses (300, 600, or 900 mg) in a double-blinded, randomized manner. Plasma concentrations of the active thiol metabolite, unchanged clopidogrel, and the inactive carboxyl metabolite of clopidogrel were determined before and serially after drug administration. Optical aggregometry was performed before and 4 hours after administration of clopidogrel. Loading with 600 mg resulted in higher plasma concentrations of the active metabolite, clopidogrel, and the carboxyl metabolite compared with loading with 300 mg (P<0.03) and lower values for adenosine diphosphate-induced (5 and 20 μmol/L) platelet aggregation 4 hours after drug administration (P=0.01 and 0.004). With administration of 900 mg, no further increase in plasma concentrations of active metabolite and clopidogrel (P≥0.38) and no further suppression of adenosine diphosphate-induced (5 and 20 μmol/L) platelet aggregation 4 hours after drug administration was achieved when compared with administration of 600 mg (P=0.59 and 0.39).

Conclusions—Single doses of clopidogrel higher than 600 mg are not associated with an additional significant suppression of platelet function because of limited clopidogrel absorption. (Circulation. 2005;112:2946-2950.)

Key Words: platelets ■ pharmacology ■ receptors ■ pharmacokinetics

In patients who undergo percutaneous coronary interventions (PCI), dual antiplatelet therapy consisting of aspirin and clopidogrel is the regimen of choice to prevent thrombotic complications. The thienopyridine clopidogrel is a prodrug that needs to be metabolized to an active compound that targets the platelet G\(_\text{i}\)-coupled adenosine diphosphate (ADP) P2Y\(_{12}\) receptor. More specifically, clopidogrel is oxidized in a cytochrome P450 (CYP) monooxygenase-dependent way to 2-oxo-clopidogrel, an intermediate metabolite that is further hydrolyzed to the active thiol metabolite of clopidogrel. The active metabolite irreversibly binds to the P2Y12 receptor. Although CYP3A4 is not the only cytochrome P450 isoenzyme involved in the metabolization of clopidogrel, it is quantitatively the most important one. The major circulating metabolite of clopidogrel is a carboxylic acid derivate that completely lacks antiaggregatory activity. We developed a method based on mass spectrometry to assess the plasma concentrations of the active metabolite of clopidogrel, unchanged clopidogrel, and the inactive carboxyl metabolite and provided the first pharmacokinetic study of clopidogrel involving its active metabolite.

For patients undergoing PCI, the administration of a clopidogrel loading dose ranging from 300 to 600 mg is recommended. Recently, we showed that administration of a 600-mg dose of clopidogrel in patients already chronically treated with clopidogrel results in a significant additional inhibition of ADP-induced platelet aggregation and ADP-induced platelet glycoprotein IIb/IIIa and P-selectin expression. This finding suggests that the degree of platelet inhibition attainable with a single 600 mg dose can be augmented and that a loading dose exceeding 600 mg may provide even more effective loading.

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In this randomized trial, we assessed the antiplatelet effects and the pharmacokinetics of 3 loading doses of clopidogrel (300, 600, and 900 mg).

Methods

Patients

Eligible for this double-blinded, randomized trial were patients with suspected or documented coronary artery disease admitted to our hospital for coronary angiography. Patients with unstable angina, acute myocardial infarction, hemodynamic instability, stroke within 3 months, malignancies, active bleeding and bleeding diatheses, oral anticoagulation therapy with a coumarin derivate, recent treatment (less than 30 days) with a glycoprotein IIb/IIIa antagonist or other antiplatelet drugs except for aspirin, platelet count \(<150 \times 10^9/L\), a serum creatinine level >2 mg/dL, and liver disease resulting in a bilirubin level >2 mg/dL were excluded. The study protocol was approved by the institutional ethics committee, and patients gave written informed consent for participation.

Randomization, Administration of Clopidogrel, and Blood Sampling

Patients eligible for the study were randomly assigned to 1 of the 3 loading doses. To enable double-blinded randomization, clopidogrel tablets (4, 8, or 12) were crushed and filled in vials made of brown glass. Mannitol was added to achieve the same volume of powder in each vial. Vial contents were diluted with 50 mL of water and then ingested. The clopidogrel-containing vials and the randomization sequence were provided by the pharmacy of the Deutsches Herzzentrum, Munich, Germany. In addition to the randomized study medication, each patient received 100 mg of aspirin. Peripheral venous blood samples were drawn in a fasting state with a loose vein. A multiple syringe sampling technique was used, and the first 2 mL of blood was discarded. For optical aggregometry, peripheral venous blood was collected in 3.8% citrate immediately before and 10 minutes and stored at \(-70^\circ C\).

Aggregometry

Citrated blood samples for aggregometry were processed within 60 minutes. Platelet aggregation was evaluated by optical aggregometry in platelet-rich plasma, using a Chrono-log lumi-aggregometer (Probe & Go Labordiagnostica) with a constant stirring rate of 1000 rpm at 37°C. The final platelet count was adjusted to 300\times10^9/L with autologous platelet-poor plasma. Platelet-rich plasma (0% light transmission) and platelet-poor plasma (100% light transmission) served as references. After baseline adjustment, ADP (final concentration, 5 or 20 \(\mu\)mol/L) was added and aggregation was recorded for 5 minutes. The analyzed parameter was maximal aggregation (%).

Detection of Clopidogrel and Its Metabolites in Plasma

Analysis was performed under modification of our previously described method on a triple-quadrupole tandem mass spectrometer (TSQ Quantum, Thermo Electron) equipped with a thermostat (5°C) Surveyor autosampler and a thermostat (50°C) Surveyor high-performance liquid chromatography system (Thermo Electron) operating in positive electrospray ionization (ESI) mode. Briefly, 15-\(\mu\)L aliquots of acetonitrile-precipitated plasma samples were injected onto a 5-\(\mu\)m Kromasil C18 column (100×3 mm; Thermo Electron) and eluted isocratically at a flow rate of 0.3 mL/min (run time, 3.5 minutes). The mobile phase consisted of 90% (vol/vol) acetonitrile/0.1% formic acid, and 10% (vol/vol) deionized water/0.1% formic acid. Clopidogrel, the inactive carboxyl metabolite, and the active thiol metabolite were identified in plasma by the specific collision-induced dissociation product ions of the respective parent isotopic \(^{3}Cl\text{ and }^{35}Cl\text{ ions [M–H]}^{\text{−}}\). Precursor ion [M–H]– produces single reaction monitoring used for quantification were \(m/z\) 322\text{→}212 for unchanged clopidogrel, \(m/z\) 308\text{→}198 for the carboxyl metabolite, and \(m/z\) 356\text{→}212 for the active metabolite (collision energy, 25 eV). Detection of the internal standard 1-methyl-4-phenylpyridinium bromide (5 mg/L) was performed by monitoring the \(m/z\) 170\text{→}127 transition (collision energy, 25 eV). The method was validated according to good laboratory practice standards. For clopidogrel determination, the intra-assay and interassay coefficients of variation were 5.8% and 8.9%, respectively. For the active metabolite (considering the calibration curve of clopidogrel and plasma standards of patients under clopidogrel therapy instead of QC's), the respective coefficients were 6.9% and 9.9% and for the carboxyl metabolite 5.5% and 8.4%.

End Points and Sample Size Calculation

The primary end point of the study was maximal ADP-induced (5 \(\mu\)mol/L) platelet aggregation 4 hours after administration of clopidogrel. To calculate the sample size, we used the method appropriate for more than 2 groups described by Lachin. It was hypothesized that maximal ADP-induced (5 \(\mu\)mol/L) platelet aggregation after administration of 300, 600, and 900 mg of clopidogrel is 60±15%, 50±15%, and 40±15% (mean±SD), respectively. Choosing a power of 90% and a 2-sided \(\alpha\)-level of 0.025 (the usual \(\alpha\)-level of 0.05 corrected for the 2 planned comparisons according to the Bonferroni method), we calculated that a sample size of at least 18 in each group was required (nQuery advisor, version 4.0, Statistical Solutions). Additional end points were the maximal plasma concentrations of clopidogrel and its metabolites.

Statistical Analysis

Data are presented as mean±SD, mean±SEM, counts, or percent ages. Frequencies of categorical variables were compared among treatment groups with a \(\chi^2\) test or Fisher’s exact test as appropriate. Means of continuous variables were compared among treatment groups with 1-way ANOVA and Student’s \(t\) test. Repeated pharmacokinetic observations over time were analyzed by using repeated-measures ANOVA with contrasts for the 3 different clopidogrel doses.

Results

Major baseline characteristics of the patients according to clopidogrel loading dose are displayed in Table 1. Concomitant medication with aspirin, \(\beta\)-blockers, angiotensin-converting enzyme inhibitors, and statins is shown in Table 2.

Figure 1 shows serial plasma concentrations for active metabolite, unchanged clopidogrel, and carboxyl metabolite

### TABLE 1. Baseline Characteristics of Patients According to Clopidogrel Loading Dose

<table>
<thead>
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<th>300 mg</th>
<th>600 mg</th>
<th>900 mg</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>64.1±11.0</td>
<td>66.0±11.8</td>
<td>64.1±10.1</td>
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<tr>
<td>Female</td>
<td>4 (20)</td>
<td>7 (35)</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Weight</td>
<td>82.2±13.1</td>
<td>78.3±13.0</td>
<td>81.3±14.7</td>
</tr>
<tr>
<td>Height</td>
<td>171±9.3</td>
<td>169±7.9</td>
<td>170±10.0</td>
</tr>
<tr>
<td>Platelet count, (10^9/L)</td>
<td>230±41</td>
<td>206±39</td>
<td>199±38</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>18 (90)</td>
<td>19 (95)</td>
<td>18 (90)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>12 (60)</td>
<td>13 (65)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Active smoker</td>
<td>2 (10)</td>
<td>2 (10)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (20)</td>
<td>3 (15)</td>
<td>3 (15)</td>
</tr>
</tbody>
</table>

Data are mean±SD or numbers of patients (percentages).
after administration of clopidogrel. Loading with 600 mg resulted in higher plasma concentrations of active metabolite, clopidogrel, and carboxyl metabolite compared with loading with 300 mg (P=0.03). With administration of 900 mg, no further increase in plasma concentrations of active metabolite and clopidogrel (P=0.38) was achieved.

Before administration of clopidogrel, there were no significant differences in maximal ADP-induced aggregation between the groups treated with 300, 600, and 900 mg (89.9±9.8%, 86.9±13.6%, and 88.6±9.3%; P=0.70 when stimulated with 5 μmol/L, and 98.6±6.9%, 96.2±11.7%, and 100.3±8.5%; P=0.40 when stimulated with 20 μmol/L). When induced with 5 μmol/L ADP, maximal aggregation after administration of clopidogrel was 66.5±18.0% (300 mg), 52.7±14.9% (600 mg), and 49.7±19.0% (900 mg), as demonstrated in Figure 2A. ADP-induced (20 μmol/L) maximal aggregation 4 hours after clopidogrel loading was 85.1±14.2% (300 mg), 69.8±16.6% (600 mg), and 64.8±18.9% (900 mg), as shown in Figure 2B. As shown in Figure 2A and 2B, although there was a greater suppression of ADP-induced platelet aggregation with 600 mg compared with 300 mg clopidogrel, no further significant enhancement of suppression was observed with 900 mg clopidogrel. In all patients, cmax of the active metabolite correlated with the absolute reduction of maximal ADP-induced (5 and 20 μmol/L) aggregation (r=0.50 and r=0.56, respectively).

### Discussion

To our knowledge, this is the first study comparing the antiplatelet effects and pharmacokinetics of different clopidogrel loading doses. The most important result of this study is that an increase of the clopidogrel loading dose from 600 to 900 mg does not result in further suppression of ADP-induced platelet aggregation caused by a failed increase in plasma concentration of the active metabolite and the unchanged form of the drug. This suggests that intestinal absorption becomes the bottleneck when single doses exceeding 600 mg are administered.

In addition, the results of this study show that there is an increase in plasma concentrations of clopidogrel compounds when 600 mg clopidogrel is given instead of 300 mg. This is associated with a substantial enhancement of platelet inhibition with the 600-mg dose compared with the 300-mg dose. Thus, the results of this trial confirm an earlier study in which the 600-mg loading dose was also more effective in suppressing platelet aggregation 4 hours after drug administration than the 300-mg loading dose.14

Earlier studies on the pharmacodynamics of single doses of clopidogrel showed that the vast majority of the antiplatelet effect of single doses (up to 600 mg) can be recorded within 2 hours.15,16 This observation is consistent with time to reach cmax (tmax) values of clopidogrel-related compounds, including the active metabolite in the order of 1 hour.7,8 The full antiplatelet effect of a 600-mg loading dose in patients scheduled for PCI occurs within 2 to 3 hours.14,17 In this study, postdose aggregometry was performed 4 hours after administration of the loading dose. Even in the group loaded with 900 mg, the peak plasma concentration of the active metabolite was still reached within less than 1 hour.

Failed increase in absorption with 900 mg clopidogrel prevented the evaluation of the level in which saturation of metabolism would have occurred. Individual patients may show limited ability to metabolize clopidogrel even for a 600 mg dose.18

In a previous study, we have shown that administration of a loading dose of 600 mg clopidogrel in patients receiving chronic clopidogrel therapy achieves a stronger platelet inhibition than that observed after 600 mg clopidogrel in first

### Table 2. Concomitant Medication of Patients

<table>
<thead>
<tr>
<th></th>
<th>300 mg n=20</th>
<th>600 mg n=20</th>
<th>900 mg n=20</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>14 (70)</td>
<td>14 (70)</td>
<td>15 (75)</td>
<td>0.92</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>12 (60)</td>
<td>15 (75)</td>
<td>12 (60)</td>
<td>0.52</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>11 (55)</td>
<td>11 (55)</td>
<td>11 (55)</td>
<td>1.00</td>
</tr>
<tr>
<td>Statins</td>
<td>9 (45)</td>
<td>8 (40)</td>
<td>8 (40)</td>
<td>0.93</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Simvastatin</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Data are numbers of patients (percentages). ACE indicates angiotensin-converting enzyme.

![Figure 1. Plasma concentrations of the active metabolite (A), clopidogrel (B), and the carboxyl metabolite (C) before and serially after administration of 300 (blue), 600 (red), and 900 (cyan) mg of clopidogrel. Data are presented as mean±SEM and analyzed by repeated-measures ANOVA with contrasts for the 3 different clopidogrel doses.](http://circ.ahajournals.org/content/113/21/e2948/F1)

![Image](314x407 to 548x726)
Second, we did not collect data on platelet function earlier than 4 hours (eg, 1 or 2 hours) after drug intake. Therefore, we cannot answer the question of whether platelet function inhibition occurs earlier with the 900-mg dose than with the 600-mg dose, although a faster onset of the antiplatelet effect with the 900-mg dose appears unlikely in the light of the pharmacokinetic data.

These data support the use of 600 mg as a loading dose in patients undergoing PCI, although randomized trials with clinical end points are needed to establish the optimal clopidogrel loading dose in these patients. A pretreatment strategy based on the 600-mg dose has been shown to provide sufficient protection in patients with stable coronary artery disease, avoiding the need for additional glycoprotein IIb/IIIa antagonists.21,22

In conclusion, our findings indicate that single doses of clopidogrel higher than 600 mg are not associated with significantly enhanced suppression of platelet function due to limited clopidogrel absorption.

Acknowledgments

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References


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