Time Dependence of Platelet Inhibition After a 600-mg Loading Dose of Clopidogrel in a Large, Unselected Cohort of Candidates for Percutaneous Coronary Intervention

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Background—Pretreatment with clopidogrel can reduce the risks associated with percutaneous coronary intervention (PCI). To shorten the time for clopidogrel to become effective, a 600-mg loading dose has been used. We sought to validate this regimen in a large cohort and investigated the time dependence of the antiplatelet effect of 600 mg of clopidogrel.

Methods and Results—Our study included 1001 patients who were scheduled for cardiac catheterization as potential candidates for PCI. We obtained blood samples before administration of 600 mg of clopidogrel and at the time of catheterization, which varied according to logistic needs. We assessed platelet aggregation by optical aggregometry and surface expression of P-selectin and activated glycoprotein IIb/IIIa by flow cytometry. Platelet aggregation induced by 5 μmol/L ADP was 51%1100614% when catheterization was performed at <1 hour, 41±14% at 1 to <2 hours, 37±15% at 2 to <4 hours, 36±13% at 4 to <6 hours, and 35±14% at ≥6 hours after clopidogrel administration. After 2 hours (n=718), the level of platelet aggregation and the surface expression of P-selectin and activated glycoprotein IIb/IIIa did not change significantly with time after clopidogrel (P>0.24 by univariate or multivariate regression). Comedication with CYP3A4 metabolized statins did not significantly affect platelet aggregation after clopidogrel (P=0.62). Among the 428 patients undergoing PCI, the 30-day composite rate of major adverse cardiac events was 1.9%, with no significant difference between patients undergoing PCI within 2 hours after clopidogrel loading and those undergoing PCI at a later time point.

Conclusions—After loading with 600 mg of clopidogrel, the full antiplatelet effect of the drug is achieved after 2 hours. Statins do not interfere with the level of platelet inhibition after this dose. (Circulation. 2005;111:2560-2564.)

Key Words: stents • coronary disease • platelets • drugs

Effective platelet inhibition at the time of percutaneous coronary intervention (PCI) with stent placement reduces the risk of periprocedural myocardial infarction (MI) substantially. In PCI-CURE (Clopidogrel in Unstable angina to prevent Recurrent ischemic Events in patients undergoing Percutaneous Coronary Intervention), patients who had been pretreated with clopidogrel for a median of 10 days incurred fewer periprocedural MIs than patients without pretreatment, with an absolute reduction in the 30-day rate of MI of 2%.1 In CREDO (Clopidogrel for the Reduction of Events During Observation), pretreatment with a loading dose of 300 mg of clopidogrel for >6 hours reduced the combined risk of death, MI, and stroke by almost 2.5%, absolute.3 In contrast, clopidogrel administered for <6 hours was not associated with significant benefit. This observation can be explained by platelet function studies that showed a less than maximal platelet inhibition within the first 6 hours after a 300-mg dose of clopidogrel.3,4 Hence, after 300 mg of clopidogrel, at least 6 hours appears to be needed for the formation of sufficient amounts of the active compound from the prodrug clopidogrel.

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A 6-hour delay between pretreatment and PCI interferes with optimization of clinical pathways of patients undergoing PCI. To overcome this problem, a 600-mg loading dose of clopidogrel has been proposed. Platelet function assays in 10 patients revealed that inhibition of ADP-induced platelet aggregation is near maximal at 2 hours after loading with 600 mg of clopidogrel.5 Likewise, after loading with 600 mg of clopidogrel, no significant changes in activation-dependent surface molecules were found in 23 patients between 4 and 24 hours.4 These findings, however, have not been confirmed in large cohorts. There is also concern that chronic treatment...
with statins metabolized by the CYP3A4 system might interfere with clopidogrel-induced platelet inhibition.5

We therefore investigated the time dependence of the antiplatelet effects of clopidogrel and the effects of concomitant statin therapy in a large unselected cohort of patients who were potential candidates for PCI. Specifically, we tested whether there was any significant change in platelet inhibition later than 2 hours after loading with 600 mg of clopidogrel.

Methods

Patient Selection

Patients scheduled for cardiac catheterization as potential candidates for PCI were eligible for the study if they were undergoing treatment with aspirin (≥100 mg/d). We did not include patients with acute MI according to the American Heart Association/American College of Cardiology criteria, patients with chronic oral anticoagulation, patients with thienopyridine treatment within the last 2 weeks before admission, and patients with contraindication to aspirin, clopidogrel, or heparin. All patients gave written informed consent, and our institutional ethics committee approved the study.

Study Protocol and Platelet Function Assays

Immediately after inclusion in the study, blood was drawn for platelet function assays with tubes that contained 3.8% sodium-citrate (Sarstedt AG). Thereafter, patients received an oral dose of 600 mg of clopidogrel. We obtained the second blood sample at the time of catheterization before administration of heparin or contrast medium. Catheterization of the study patients was timed according to the routine schedule of the catheterization laboratory. Patients undergoing PCI were monitored subsequently by systematic serial creatinine kinase measurements and ECG recordings. Platelet function was evaluated ex vivo by optical aggregometry with 2 concentrations of ADP (5 and 20 μmol/L) as described previously6 with platelet-rich plasma adjusted to 275 to 325 10^3 platelets/μL. The coefficient of variation of our optical aggregometry assay is 6.1% after stimulation with 5 μmol/L ADP and 5.3% after stimulation with 20 μmol/L ADP (n=4 determinations in plasma from 6 subjects). As previously described, we assessed the 20 μmol/L ADP-induced surface expression of P-selectin and activated glycoprotein Ib/IIa by triple-color flow cytometry after staining the platelets with the following monoclonal antibodies: FITC (fluorescein isothiocyanate)-conjugated PAC-1 (recognizes activated GPIb/IIa receptors; Becton Dickinson), PE (phycoerythrin)-conjugated anti-CD41 (recognizes total GPIIb/IIIa receptors; Becton Dickinson), and PCS (phycoerythrin-cyanin 5.1)-conjugated anti-CD41 (recognizes total GPIb/IIa receptors; Beckman Coulter).6

Statistical Analysis

For all statistical analyses, we used the SPSS software package, version 11.0. Discrete variables are reported as counts (percentages) and continuous variables as mean±SD or number of patients (percentage).

Results

The present study included 1001 consecutive patients. Table 1 shows the baseline demographic and clinical characteristics of the study cohort. As detailed in Table 2, 539 patients were undergoing chronic treatment with statins.

By all platelet function tests (Table 3), patients who underwent catheterization within the first 2 hours after
clopidogrel administration had a lower level of platelet inhibition than patients who underwent catheterization after ≥2 hours. At all time points, we found considerable scatter in the level of platelet function as assessed by ADP-induced platelet aggregation (Figure 1, left panel), inhibition of ADP-induced P-selectin expression (Figure 1, right panel), or other platelet assays (data not shown). Figure 1 also shows the fitted functions for the exponential regression fit between time from clopidogrel loading and ADP-induced platelet aggregation (left panel) or inhibition of ADP-induced P-selectin expression (right panel).

Within the group undergoing catheterization at ≥2 hours (n=718) after administration of clopidogrel, there were no significant differences in platelet function between the different classes of time after clopidogrel bolus dose (Table 3). Likewise after 2 hours, time from clopidogrel administration showed no meaningful association with the inhibition of ADP-induced platelet aggregation (5 μmol/L) by univariate analysis (linear $r^2=0.001$, $P=0.366$, exponential decay $r^2=0.009$) or multivariate analysis (partial $r^2=0.002$, $P=0.245$). Moreover, we did not find any significant effect of statin therapy on inhibition of 5 μmol/L ADP-induced platelet aggregation (Figure 2). This was confirmed in the multivariate analyses (partial $r^2<0.001$, $P=0.915$ for inhibition of 5 μmol/L ADP-induced platelet aggregation). Likewise, within the group undergoing catheterization at ≥2 hours after administration of clopidogrel, our multivariate analyses did not show a significant relation of time after clopidogrel loading or statin medication with inhibition of either ADP-induced P-selectin expression (partial $r^2<0.001$ and 0.001, $P=0.726$ and 0.320, respectively) or activated glycoprotein IIb/IIIa (partial $r^2=0.001$ and <0.001, $P=0.381$ and 0.728, respectively). None of the other variables shown in Table 1 had an impact on platelet aggregation or surface expression of P-selectin or activated glycoprotein IIb/IIIa after clopidogrel.

Among the 428 patients undergoing PCI, the 30-day composite rate of major adverse cardiac events was 1.9% (Table 4). We did not find any significant difference with respect to major adverse cardiac events between patients undergoing PCI within 2 hours after clopidogrel loading and those undergoing PCI at a later time point (Table 4). A Thrombolysis in Myocardial Infarction (TIMI) major bleeding (intracranial hemorrhage or drop in hemoglobin of >5 g/dL, assuming an increase of 1 g/dL in hemoglobin for each unit of blood transfused) occurred in 1.2% (5/428) of the PCI group. In addition, 11 patients (2.6%) incurred false aneurysms of the access site, all of which could be managed by ultrasound-guided compression. Again, there was no significant difference between the 2 subgroups undergoing PCI before or after the 2-hour cutoff. In patients who did not undergo PCI, the rate of bleeding and access site complications was substantially lower than in those treated with PCI.
Discussion

Our study investigated the antiplatelet effects of a loading dose of 600 mg of clopidogrel with ex vivo platelet function assays in a cohort of 1001 unselected patients who were potential candidates for elective PCI. We found a time-dependent increase in the level of platelet inhibition during the first 2 hours; thereafter, the degree of platelet inhibition was independent of time after loading. Analysis of surface expression of activation-dependent glycoproteins, such as P-selectin and the activated glycoprotein IIb/IIIa, confirmed our findings obtained by optical aggregometry.

TABLE 4. Thirty-Day Rate of Major Adverse Cardiac Events After PCI and Noncardiac Complications Until Discharge

<table>
<thead>
<tr>
<th>Patients With PCI</th>
<th>Entire Cohort</th>
<th>&lt;2 Hours</th>
<th>≥2 Hours</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with PCI, n</td>
<td>428</td>
<td>135</td>
<td>293</td>
<td></td>
</tr>
<tr>
<td>Any MACE</td>
<td>1.9 (8)</td>
<td>1.5 (2)</td>
<td>2.0 (6)</td>
<td>0.78</td>
</tr>
<tr>
<td>Death</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>...</td>
</tr>
<tr>
<td>Nonfatal MI</td>
<td>1.4 (6)</td>
<td>0.7 (1)</td>
<td>1.7 (5)</td>
<td>0.49</td>
</tr>
<tr>
<td>TVR</td>
<td>1.2 (5)</td>
<td>0.7 (1)</td>
<td>1.4 (4)</td>
<td>0.64</td>
</tr>
<tr>
<td>TIMI major bleed</td>
<td>1.2 (5)</td>
<td>0.7 (1)</td>
<td>1.4 (4)</td>
<td>0.64</td>
</tr>
<tr>
<td>False aneurysm</td>
<td>2.6 (11)</td>
<td>2.2 (3)</td>
<td>2.7 (8)</td>
<td>0.87</td>
</tr>
<tr>
<td>Transfusion</td>
<td>0.2 (1)</td>
<td>0.0 (0)</td>
<td>0.3 (1)</td>
<td>0.52</td>
</tr>
<tr>
<td>Patients without PCI, n</td>
<td>573</td>
<td>156</td>
<td>417</td>
<td></td>
</tr>
<tr>
<td>TIMI major bleed</td>
<td>0.5 (3)</td>
<td>0.6 (1)</td>
<td>0.5 (2)</td>
<td>0.82</td>
</tr>
<tr>
<td>False aneurysm</td>
<td>0.3 (2)</td>
<td>0.6 (1)</td>
<td>0.2 (1)</td>
<td>0.47</td>
</tr>
<tr>
<td>Transfusion</td>
<td>0.3 (2)</td>
<td>0.6 (1)</td>
<td>0.2 (1)</td>
<td>0.47</td>
</tr>
</tbody>
</table>

MACE indicates major adverse cardiac events; nonfatal MI, nonfatal MI defined as new Q wave or rise in creatinine kinase to 3 times the upper limit of normal with concomitant rise in MB isoenzyme.

Data are expressed as percentage of subgroup (No. of patients).

*P for comparison between <2 hours and ≥2 hours.

The ISAR-REACT (Intracoronary Stenting and Antithrombotic Regimen—Rapid Early Action for Coronary Treatment) study showed that after a loading dose of 600 mg, clopidogrel alone can give sufficient protection in non–high-risk PCI. In this setting, clinical outcome was not improved by abciximab compared with placebo. In ISAR-REACT, the minimum delay after clopidogrel loading was 2 hours. Although outcome in ISAR-REACT was similar, irrespective of whether PCI was performed within 2 to 6 hours or later, there was still uncertainty whether a 2-hour wait after clopidogrel is sufficient. Only 25% of the patients underwent PCI within 2 to 4 hours after clopidogrel. In the present large cohort, we could clarify that the level of platelet inhibition could not be improved significantly by waiting longer than 2 hours after a loading dose of 600 mg of clopidogrel.

Although we did not observe any time-dependent changes beyond 2 hours, the inhibitory effect of the 600-mg loading dose of clopidogrel showed a large variability. This variation was independent of concomitant medication with statins, and there was no specific subgroup defined by demographic or clinical variables that was prone to a reduced antiplatelet effect of clopidogrel. The present findings on the lack of effect of chronic medication with statins after loading with 600 mg of clopidogrel extend earlier findings on acute statin medication in 77 patients and on chronic statin medication in 190 patients.

The high loading dose of clopidogrel used in the present study was well tolerated and was not associated with an excessive risk of vascular access site or bleeding complications. This finding is consistent with 2 earlier studies, ISAR-REACT and a registry by the same group.

Study Limitations

Timing of the second blood sample was determined by the need to prepare patients for catheterization and by the schedule of the catheterization laboratory. Because we did not randomize our patients to strata of time after clopidogrel, we need to consider potential bias in the timing of this second sample. To minimize this bias, we performed multivariate analyses that included pertinent clinical covariables. These analyses confirmed the primary observations.

The present study was not designed to investigate the relation between clinical outcome and timing of PCI after clopidogrel loading. Given the low risk for these complications after elective PCI, a larger sample size of patients with PCI than that included in the present study is needed to address this issue. Hence, we did not expect to find significant differences in outcome of patients undergoing PCI within 2 hours after clopidogrel loading compared with those undergoing PCI after ≥2 hours.

Clopidogrel is a reversible competitive inhibitor of CYP3A4. The degree of competitive inhibition between substrates depends on the relative affinity of the substrates for the binding site and their relative concentrations. Therefore, we cannot exclude that at clopidogrel loading dosages <600 mg or for higher proportions of patients with statin dosing >20 mg/d, the interaction between CYP3A4-metabolized statins and clopidogrel might be more relevant to the inhibition of platelet aggregation than in the present study.
Clinical Implications
The present study demonstrates that after a loading dose of 600 mg of clopidogrel, there is no need to delay PCI for more than 2 hours to achieve the full antiplatelet effect of this medication. Within the spectrum of a large cohort of potential candidates for elective PCI, we did not identify any clinical entity that requires special attention with respect to platelet inhibition after 600 mg of clopidogrel. Particularly, patients taking chronic medication with statins metabolized via CYP3A4 do not need to be treated differently. Given our findings, we therefore suggest a wait of at least 2 hours to ensure safe performance of PCI after a 600-mg loading dose of clopidogrel.

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References
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