Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets
Results of the Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets (CLEAR PLATELETS) Study

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Background—Pretreatment is not the most common strategy practiced for clopidogrel administration in elective coronary stenting. Moreover, limited information is available on the antiplatelet pharmacodynamics of a 300-mg versus a 600-mg clopidogrel loading dose, and the comparative effect of eptifibatide with these regimens is unknown.

Methods and Results—Patients undergoing elective stenting (n=120) were enrolled in a 2×2 factorial study (300 mg clopidogrel with or without eptifibatide; 600 mg clopidogrel with or without eptifibatide) (Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets [CLEAR PLATELETS] Study). Clopidogrel was administered immediately after stenting. Aggregometry and flow cytometry were used to assess platelet reactivity. Eptifibatide added a ≥2-fold increase in platelet inhibition to 600 mg clopidogrel alone at 3, 8, and 18 to 24 hours after stenting as measured by 5 μmol/L ADP–induced aggregation (P<0.001). Without eptifibatide, 600 mg clopidogrel produced better inhibition than 300 mg clopidogrel at all time points (P<0.001). Glycoprotein IIb/IIIa (GPIIb/IIIa) blockade was associated with lower cardiac marker release. Active GPIIb/IIIa expression was inhibited most in the groups treated with eptifibatide (P<0.05).

Conclusions—In elective stenting without clopidogrel pretreatment, use of a GPIIb/IIIa inhibitor produces superior platelet inhibition and lower myocardial necrosis compared with high-dose (600 mg) or standard-dose (300 mg) clopidogrel loading alone. In the absence of a GPIIb/IIIa inhibitor, 600 mg clopidogrel provides better platelet inhibition than the standard 300-mg dose. These results require confirmation in a large-scale clinical trial. (Circulation. 2005;111:1153-1159.)

Key Words: inhibitors ■ platelets ■ stents ■ thrombosis

We have reported response variability to clopidogrel in patients undergoing elective coronary artery stenting treated with the standard 300-mg loading dose that in turn affects posttreatment platelet reactivity.1,2 Platelet reactivity is greatest early after stenting, and it has been reported that patients with high poststent platelet reactivity may be at the greatest risk of stent thrombosis and ischemic events.3–5 These data suggest that clopidogrel therapy as administered in the current 300-mg loading dose does not provide sufficient platelet inhibition in some patients undergoing coronary stenting. Therefore, these findings have particular relevance to adjunctive glycoprotein IIb/IIIa (GPIIb/IIIa) therapy because treatment with the latter agents may overcome the limitations of clopidogrel as an antiplatelet agent early after stenting.

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The mechanisms of nonresponse to clopidogrel are incompletely defined.6 Inadequate levels of active metabolite are a potential explanation that may be overcome by higher loading doses.7 A recent report has suggested that a larger clopidogrel loading dose (600 mg) may be associated with a lower incidence of resistance.8 A small study comparing a loading regimen of 300 mg with 600 mg reported superior early inhibition with the higher dose.9 However, a large prospective pharmacodynamic comparison of a 300-mg loading dose versus a 600-mg dose is not available. In addition, the effect of adding eptifibatide, a potent GPIIb/IIIa inhibitor, to the aforementioned regimens has never been prospectively investigated.

The recent Intracoronary Stenting and Antithrombotic Regimen–Rapid Early Action for Coronary Treatment (ISAR-REACT) study has suggested that pretreatment with 600 mg clopidogrel provides outcomes similar to those achieved with a strategy of pretreatment with the addition of abciximab.10 However, an important consideration in the decision for pretreatment is the associated risk of bleeding in...
those patients requiring coronary bypass surgery.11,12 Moreover, data from the first 6 months of 2004 in the CRUSADE (Can Rapid Risk Stratification of Unstable Angina Patients Suppress Adverse Outcomes with Early Implementation of the ADD/AHA Guidelines) Registry demonstrate that 90% of patients receive 300 mg clopidogrel as their loading dose, and only the minority are pretreated (oral communication, Eric Peterson, MD, Duke University, Durham, NC, November 2004). Finally, little is known of the relationship between poststent platelet reactivity and the occurrence of myocardial necrosis.

Therefore, we performed a prospective randomized pharmacodynamic investigation of 4 antiplatelet regimens to address these limitations: (1) clopidogrel 300 mg; (2) clopidogrel 600 mg; (3) clopidogrel 300 mg plus eptifibatide; and (4) clopidogrel 600 mg plus eptifibatide (Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets [CLEAR PLATELETS] Study). Clopidogrel was administered immediately after stenting. The primary aim was to compare the antiplatelet effects of these regimens. A secondary aim was to study the relationship between platelet reactivity in each group and the occurrence of postprocedural myocardial necrosis. On the basis of the available data, we hypothesized that (1) the antiplatelet effects of eptifibatide administered for coronary stenting with either loading dose of clopidogrel will be associated with superior platelet inhibition compared with either loading dose of clopidogrel alone, and (2) high-dose clopidogrel alone will provide superior platelet inhibition compared with the standard loading dose alone.

Methods

The investigational review board approved this study. Consecutive patients undergoing elective coronary stenting were enrolled after giving informed consent. Patients were aged >18 years. The exclusion criteria were as follows: a history of bleeding diathesis, acute myocardial infarction within 48 hours, elevated cardiac markers (above upper limits of normal for the respective assay), cerebrovascular event within 3 months, chronic vessel occlusion or angiographically visible thrombus, illicit drug or alcohol abuse, prothrombin time >1.5 times control, platelet count <100 000/mm³, hematocrit <30%, creatinine >4.0 mg/dL, and thienopyridine or GPIIb/IIIa use before the procedure.

Patients were randomly assigned to 1 of 4 treatment regimens by a computer-generated assignment that was chosen from a sealed envelope by the study personnel: group A, clopidogrel 300 mg; group B, clopidogrel 600 mg; group C, clopidogrel 300 mg plus eptifibatide; and group D, clopidogrel 600 mg plus eptifibatide. The clopidogrel loading dose was given to all patients immediately after stenting and was followed by 75 mg daily. In addition, all patients had received at least 81 mg aspirin for 7 days before the procedure (>90% received 325 mg), and 325 mg was administered on the day of the procedure and daily thereafter. Eptifibatide was administered according to the Enhanced Suppression of the Platelet IIb/IIIa Receptor With Integrilin Therapy (ESPRIT) study protocol as a computerized method to give the loading dose immediately after the procedure.
double bolus (180 μg/kg) followed by an infusion (2 μg/kg per minute) for 18 to 24 hours after the procedure. Unfractionated heparin was administered according to the ESPRIT dosing regimen (60 U/kg) as a bolus to all patients in the catheterization laboratory immediately before stenting.

**Blood Sampling**

Baseline blood samples were obtained in the catheterization laboratory through the indwelling femoral vessel sheath and transferred to Vacutainer blood-collecting tubes (Becton-Dickinson) containing 3.8% trisodium citrate after the first 2 to 3 mL of free-flowing blood was discarded. The Vacutainer tube was filled to capacity and gently inverted 3 to 5 times to ensure complete mixing of the anticoagulant. Samples were obtained before clopidogrel, eptifibatide, and heparin administration (baseline) and at 3, 8, and 18 to 24 hours after stenting. The 18- to 24-hour blood draw was performed at the time of completion of the eptifibatide infusion.

**Platelet Aggregation**

The blood-citrate samples were centrifuged at 120g for 5 minutes to recover platelet-poor plasma. The platelet count was determined in the platelet-rich plasma sample and adjusted to 3.0×10⁶/mL with homologous platelet-poor plasma. The platelet-rich plasma and platelet-poor plasma were stored at room temperature to be used within 2 hours. Platelet aggregation was assessed as described previously. Briefly, platelets were stimulated with 5 and 20 μmol/L ADP, and the aggregation was assessed with the use of a Chronolog Lumi-Aggregometer (model 490-4D) with the Aggregolink software package (Chronolog). Aggregation was expressed as the maximum percent change in light transmittance from baseline, with platelet-poor plasma used as a reference.

**Whole Blood Flow Cytometry**

The surface expression of platelet receptors was determined by whole blood flow cytometry with the use of the 3-color analysis method (Immunocytometry Systems, Cytometry Source Book, Becton Dickinson) with the following monoclonal antibodies: FITC-conjugated PAC-1 (recognizes activated GPIIb/IIIa receptors), R-Phycocerythrin (R-PE)—conjugated CD41a (recognizes total GPIIb/IIIa receptors), and CY-Chrome–conjugated CD62P (recognizes P-selectin). All 3 antibodies were purchased from BD Biosciences, San Diego, Calif. The blood-citrate mixture was stimulated with 5 μmol/L ADP for 2 minutes. Saturating concentrations of respective antibodies were then added, and the tubes were incubated at room temperature for 20 minutes in the dark. The labeled samples were fixed by the addition of 1% buffered paraformaldehyde and stored at 4°C for at least 2 hours. The labeled samples were analyzed by a Becton Dickinson FACScan flow cytometer set up to measure fluorescence light scatter. The instrument was calibrated with fluorescence beads for the 3-color flow cytometer setup (CaliBRITE3, BD Biosciences). After the gate was set around platelets, FL1 (FITC)/FL2 (R-PE) and FL2 (R-PE)/FL3 (CY-Chrome) compensations were adjusted. All the variables were collected by use of 4-decade logarithmic amplification. The data were collected in list mode and then analyzed with the use of CELL Quest software (BD Biosciences). P-selectin was expressed as percent positive cells (i.e., the ratio of CD62P [CY-Chrome] versus CD41a [R-PE] positive cells) as previously described. Activated GPIIb/IIIa was expressed as log mean fluorescence intensity (MFI).

**Myocardial Necrosis Markers**

Cardiac markers were measured at the same times as the platelet assays. The peak levels of troponin I, creatinine kinase MB (CK-MB), and myoglobin were determined with the use of the Triage Cardiac Panel with a Triage Meter (Biosite Inc). This method is based on a fluorescence immunoassay for the quantitative determination of these cardiac markers. The upper limit of normal value for troponin I is 1.0 ng/mL; for myoglobin is 107 ng/mL; and for CK-MB is 4.3 ng/mL.

**Definitions**

Relative platelet inhibition was defined as follows: (baseline aggregation–posttreatment aggregation)/baseline aggregation.
tion × 100%. Mean platelet reactivity was calculated as the average platelet aggregation recorded at 3, 8, and 18 to 24 hours after stenting. Relative inhibition of active GPIIb/IIIa expression was defined as follows: (baseline MFI—posttreatment MFI/baseline MFI). The definition of an infarct was CK-MB >3 times the upper limit of normal in at least 2 samples, and a large infarct was defined as CK-MB >5 times the upper limit of normal in at least 2 samples. Bleeding was quantified according to the Thrombolysis in Myocardial Infarction (TIMI) criteria. In brief, minor bleeding was defined as clinically overt bleeding accompanied by a fall in hemoglobin of 3 to ≤5 g/dL, and major bleeding occurred when the hemoglobin decreased to >5 g/dL.

Sample Size and Statistical Analysis

Previous studies from our laboratory had shown that a 300-mg clopidogrel loading dose produces <40% inhibition of baseline aggregation in response to 5 and 20 μmol/L ADP at 24 hours after administration. Other studies from our laboratory have demonstrated >80% inhibition by eptifibatide. According to the statistical calculation 2 = 2 × [Z(1−α/2) + Z(1−β)]2/Δ2, where m = number of patients, statistical significance level (α) = 5%, power (β) = 90%, and Δ = standardized difference, ~30 patients will be needed in each arm in the study.

Comparisons were made between groups by 1-way ANOVA (Statistica software). The Wilks-Shapiro test was used to assess conformity with a normal distribution. On the basis of the normal distribution of data, the mean±SEM is reported except as otherwise noted, and P<0.05 was considered significant.

Results

Patients

One hundred twenty patients were enrolled and had platelet assays performed. All of the procedures performed on the patients were elective. Eighteen patients were admitted with unstable angina (TIMI risk score ≤3). Four patients, 1 in each group, presented with non–ST-segment elevation myocardial infarction >48 hours before randomization. The remainder of the patients had stable angina. The patient demographics and procedural characteristics of the 4 treatment groups are shown in Tables 1 and 2, respectively. Group A was the oldest, and group C had the highest percentage of males. Cardiovascular risk factors were common, and the incidence of diabetes was high in all groups. One patient in each group had presented with a non–ST-segment elevation myocardial infarction. The use of statins metabolized by the CYP 3A4 pathway was the lowest in group D. Concomitant medications were frequently used in all groups. Multivessel interventions were commonly performed, and drug-eluting stents were often used.

There were no in-hospital deaths. There was 1 ST-segment elevation myocardial infarction that occurred in-hospital after a subacute thrombosis in a patient assigned to group A. There were no strokes or episodes of congestive heart failure. Hematomas were the cause of all bleeding episodes. Minor bleeding occurred in 1 patient in group A, and major bleeding occurred in 1 patient each in groups C and D.

Platelet Aggregation

Figure 1A shows the pharmacodynamic responses in the 4 groups in response to 5 μmol/L ADP. In the groups not treated with eptifibatide, baseline aggregation was 63±11% in group A and 66±7% in group B (P=NS). In the groups treated with eptifibatide, baseline aggregation was 58±11% in group C and 62±7% in group D (P=NS). In the groups not treated with eptifibatide, a 600-mg loading dose of clopidogrel provided greater platelet inhibition throughout the first 24 hours after stenting. Group B had greater inhibition than group A at 3 hours (P<0.001), 8 hours (P<0.001), and 18 to 24 hours (P<0.001). The peak inhibitory effect after a 600-mg loading dose occurred at 8 hours compared with 18 to 24 hours after a 300-mg loading dose. Groups C and D exhibited the same inhibition (P=NS at all times), and both groups exhibited ≥2-fold greater inhibition compared with groups A and B at all times (P<0.001).

Figure 1B shows the platelet response to 20 μmol/L ADP. Baseline aggregation did not differ between groups. The groups receiving clopidogrel and eptifibatide had consistently the lowest reactivity over 24 hours (P<0.001 versus groups A and B at all times). At an agonist concentration of 20 μmol/L, group D showed greater inhibition at 18 to 24 hours than group C (P=0.05). Group B had the same inhibition as group A at 3 hours (P=0.55) and greater inhibition at 8 hours (P=0.09) and 18 to 24 hours (P=0.01). The peak inhibitory effect after a 300- or 600-mg loading dose was reached at 8 hours.

Flow Cytometry

Stimulated P-selectin expression at baseline did not differ between groups. Posttreatment P-selectin expression was significantly reduced in all groups compared with baseline expression, whereas treatment with 300 mg clopidogrel alone
had the least effect in P-selectin expression (Figure 2).
Stimulated expression of active GPIIb/IIIa measured at 18 to 24 hours was inhibited the most in groups C (76 ± 13\%) and D (77 ± 5\%) compared with groups A (50 ± 20\%; \(P < 0.05\)) and B (63 ± 7\%; \(P < 0.05\)) (Figure 3).

Myocardial Necrosis Markers
Overall, CK-MB release (>1 to 3 times the upper limit of normal) was lowest in the groups treated with eptifibatide (\(P < 0.005\)) (Figure 4 and Table 3). Criteria were met for a myocardial infarction in 3 patients from group A and 1 from group B (\(P < 0.03\) for eptifibatide versus clopidogrel alone). There were no large infarcts in either group that received eptifibatide, whereas 2 occurred in group A and 1 in group B. Similar findings were observed when troponin and myoglobin were measured (Figure 5). A trend to less troponin elevation was observed in group B compared with group A (\(P = 0.10\)). Inhibition of either troponin I or myoglobin release was lower in patients treated with eptifibatide plus clopidogrel compared with clopidogrel alone (\(P = 0.004\) and \(P = 0.002\), respectively) (Figure 5).

Relation of Platelet Reactivity to Myocardial Necrosis
Figure 6 demonstrates that eptifibatide use was associated with the lowest mean platelet reactivity, and 300 mg clopidogrel alone was associated with higher mean platelet reactivity than 600 mg clopidogrel alone. Figure 7 demonstrates the overall relation of myocardial necrosis marker release to mean platelet reactivity. Mean platelet reactivity was sig-
ificantly higher in those patients who developed a myocardial infarction or any increased myocardial marker release. Myocardial infarction only occurred in those patients with mean 5 \(\mu\)mol/L ADP-induced aggregation >50\%.

Discussion
The present study demonstrates that in patients not receiving a parenteral GPIIb/IIIa blocker, a strategy of a 600-mg loading dose of clopidogrel administered at the time of elective coronary stenting provides superior platelet inhibition compared with the standard 300-mg loading dose. This effect occurs within 3 hours and persists for 24 hours after the procedure. When administered alone, a 600-mg loading dose strategy is also associated with a trend to lower occurrence of myocardial necrosis release compared with a 300-mg dose. Our study also demonstrates that the administration of eptifibatide with either a standard or high loading dose of clopidogrel provides the most sustained platelet inhibition and is associated with the lowest incidence of myocardial necrosis marker release. The incremental increases in cardiac marker release observed in patients with higher levels of posttreatment platelet reactivity lend strong support to the concept that reactive platelets play a central role in the mediation of poststent myocardial necrosis.\(^9\) The absence of infarcts in patients with <50\% mean 5 \(\mu\)mol/L ADP-induced aggregation suggests a threshold effect and may be a therapeutic target for future investigations in elective coronary stenting.

The data from the present study are consistent in part with those of a smaller study by Müller et al.,\(^9\) who reported superior early (4 hours after the procedure) inhibition with

### Table 3. Necrosis Markers

<table>
<thead>
<tr>
<th>Markers</th>
<th>Group A (n=30)</th>
<th>Group B (n=30)</th>
<th>Group C (n=30)</th>
<th>Group D (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB (&gt;1–3×ULN), n</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CK-MB (&gt;3×ULN), n</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TN-I (&gt;ULN), n</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Myoglobin (&gt;2×ULN), n</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

ULN indicates upper limit of normal; TN, troponin.
600-mg clopidogrel loading compared with 300-mg loading. However, we observed maximum inhibition at 8 hours after the procedure in the present study. Moreover, we observed not only superior early inhibition but also superior consistent inhibition over 24 hours after the high loading dose. For this reason, we believe that all pharmacodynamic studies examining clopidogrel loading strategies in stenting should include serial analyses over at least an 18- to 24-hour time period.

There have been no previous pharmacodynamic investigations examining the effects of high clopidogrel loading doses with eptifibatide. Our study suggests that 600 mg clopidogrel may also add to the antiplatelet effect of eptifibatide at 18 to 24 hours when we observed lower aggregation after stimulation with a high concentration of agonist. The recent ISAR-REACT study compared the effect of adding abciximab to 600 mg clopidogrel versus a strategy of treatment with 600 mg clopidogrel alone in patients undergoing elective stenting.10 In ISAR-REACT, patients belonged to a lower-risk group that was pretreated with clopidogrel at a median of 7.4 hours before the procedure, whereas in our study patients did not receive pretreatment. The higher risk profile of our patients and the absence of pretreatment may help to explain the overall higher infarction rate that we observed in the patients not treated with eptifibatide (≈7% versus 4%).

The ISAR-REACT investigators reported the same incidence of ischemic events in both groups, whereas the group receiving the GPIIb/IIIa inhibitor had more bleeding.10 However, the findings of ISAR-REACT assume less relevance when physicians decide not to pretreat patients whose coronary anatomy is unknown before the stent procedure. This decision is most often based on the concern for serious bleeding that has been reported in patients receiving clopidogrel within 5 to 7 days of undergoing coronary artery bypass surgery.11,12 Anatomy was rarely known in our patient population before the procedure. The relevance of our study to current practice in the United States is demonstrated by recent data from the first 6 months of 2004 in the CRUSADE Registry. This registry demonstrated that most interventional cardiologists perform procedures without clopidogrel pretreatment, and most do not administer a high loading dose (oral communication, Eric Peterson, MD, Duke University, Durham, NC, November 2004). Whether pretreatment with a high loading dose of clopidogrel would obviate the beneficial effects of parenteral GPIIb/IIIa blockade observed in our study is unknown. At the minimum, our study suggests that in the setting of elective stenting when pretreatment with clopidogrel is not administered, using a parenteral GPIIb/IIIa inhibitor with a high clopidogrel loading dose administered as soon as possible is associated with a low incidence of myocardial infarction and excellent myocardial protection, as evidenced by low overall release of cardiac markers. The definition of large infarcts used in our study has been correlated with mortality in previous investigations.20–22

Study Limitations
Although our study is the largest prospective pharmacodynamic investigation of 2 doses of clopidogrel that also includes an assessment of a GPIIb/IIIa inhibitor, it nevertheless is a small clinical trial. We observed major bleeding in 1 of 30 patients in each of the eptifibatide groups and 1 stent thrombosis in the group treated with 300 mg clopidogrel alone. However, conclusions about the relation of platelet reactivity to clinical outcomes such as bleeding, infarction, and stent thrombosis are limited and would require a much larger investigation. Similarly, conclusions about the long-term risk/benefit analysis of bleeding versus a reduction in myocardial necrosis cannot be determined from this pharmacodynamic study.

Conclusions
In conclusion, platelet reactivity plays an important role in the pathogenesis of myocardial infarction after elective coronary stenting. When there is no clopidogrel pretreatment, a strategy of parenteral GPIIb/IIIa inhibitor administration is asso-
associated with superior platelet inhibition and lower cardiac marker release compared with a strategy of high-dose (600 mg) or standard-dose (300 mg) clopidogrel loading alone. In the absence of a GPIIb/IIIa blocker, there is greater platelet inhibition with the 600-mg compared with the standard 300-mg dose. Our data support a future larger clinical study to investigate whether 600 mg should become the new standard loading strategy for this drug in elective coronary stenting.

Acknowledgments
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References
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