Loading With 600 mg Clopidogrel in Patients With Coronary Artery Disease With and Without Chronic Clopidogrel Therapy

Adnan Kastrati, MD; Nicolas von Beckerath, MD; Alexander Joost; Gisela Pogatsa-Murray, MD; Olga Gorchakova, MD; Albert Schömig, MD

Background—It is not known whether further suppression of platelet function can be achieved with clopidogrel beyond that provided by currently recommended loading and maintenance doses. We performed a comparative assessment of the antiplatelet effects of a 600-mg loading dose of clopidogrel given to patients with and without chronic clopidogrel therapy.

Methods and Results—Those eligible for this prospective study were aspirin-treated patients with suspected or documented coronary artery disease admitted to hospital for coronary angiography. Two series of 20 consecutive patients each were assessed in this study. The first series included patients who had never received clopidogrel (first-use group); the second series included patients on chronic therapy with a daily dose of 75 mg clopidogrel for ≥1 month (chronic therapy group). Blood samples were drawn before and 6 hours after oral administration of 600 mg clopidogrel for aggregometry and flow cytometry studies. In the first-use group, loading with 600 mg clopidogrel inhibited ADP 5 μmol/L-induced platelet aggregation from 90±9% to 51±19% (P<0.001). In the chronic therapy group, loading with 600 mg clopidogrel yielded further inhibition of ADP 5 μmol/L–induced platelet aggregation in addition to that achieved by the maintenance dose of 75 mg/d, from 52±14% to 33±12% (P<0.001). In both groups, 600 mg clopidogrel loading significantly inhibited ADP-induced expression of glycoprotein IIb/IIIa and P-selectin receptors.

Conclusions—Further platelet inhibition can be achieved with clopidogrel in addition to that provided by currently recommended loading and maintenance doses. Higher doses may be warranted after assessment of their clinical efficacy and safety. (Circulation. 2004;110:1916-1919.)

Key Words: angina ◊ inhibitors ◊ platelets ◊ thrombosis

Clopidogrel has become a mainstay of the pharmacological therapy for patients with atherosclerotic cardiovascular disease.1,2 especially in those undergoing percutaneous coronary interventions.2,3 Despite the widespread use of clopidogrel, several aspects of its pharmacokinetics, optimal dosing and length of therapy, and its drug interactions are still unclear.4,5 A loading dose of 600 mg clopidogrel is associated with a rapid platelet-inhibitory effect comparable to that seen in patients on chronic clopidogrel therapy.6 When given before percutaneous coronary intervention, loading with 600 mg clopidogrel obviates the need for glycoprotein (GP) IIb/IIIa inhibitors in patients with low to moderate risk.7 A clopidogrel dose of 75 mg/d is commonly used during chronic therapy. This dose has been selected because it leads to antiplatelet effects equivalent to those of 500 mg/d ticlopidine, the first of the thienopyridine drugs used clinically.8 Nevertheless, it is still not known whether further suppression of platelet function can be achieved with clopidogrel in addition to that provided by the currently recommended maintenance dose of 75 mg/d and loading dose of 300 to 600 mg. To address this question, we performed a comparative assessment of the antiplatelet effects of a 600-mg loading dose of clopidogrel given to patients with coronary artery disease with and without ongoing chronic clopidogrel therapy.

Methods

Patients
Those eligible for this prospective study were patients with suspected or documented coronary artery disease admitted to hospital for coronary angiography who had been treated chronically with aspirin (200 mg/d for ≥1 month before entry into the study). Patients with unstable angina, acute and recent (<14 days) myocardial infarction, hemodynamic instability, stroke within 3 months, malignancies, active bleeding or bleeding diathesis, oral anticoagulation therapy with a coumarin derivate, recent treatment (<30 days) with a GP Ib/IIa antagonist, platelet count <100×109/μL, a serum creatinine level >2 mg/dL, and/or liver disease resulting in a bilirubin level >2 mg/dL were excluded. Two groups of patients were enrolled. The first group included 20 patients who had never received clopidogrel treatment (first-use group); the second group included 20 patients who were already on chronic therapy with a daily dose of 75 mg.
clopidogrel for ≥1 month (chronic therapy group). The 2 cohorts were recruited in parallel on the basis of a single study design. In each cohort, patients were enrolled sequentially as they came and as they met the prespecified criteria of the first-use or chronic therapy group. In the first-use group, the first patient was enrolled in November 2003; the last patient, in March 2004. In the chronic therapy group, the first patient was enrolled in October 2003; the last patient, in February 2004. The institutional ethics committee approved the study protocol, and patients gave written informed consent for participation.

**Study Protocol**

All studies were performed in patients in a fasting state and before any invasive procedure such as coronary angiography. Peripheral venous blood samples were drawn with a loose tourniquet to avoid artifacts through a short venous catheter inserted into a forearm vein before and 6 hours after oral administration of the 600-mg loading dose of clopidogrel. A multiple-syringe sampling technique was used, and the first 2 mL blood was discarded. Blood was collected in 3.8% citrate. Samples were processed within 1 hour after blood drawing by operators unaware of whether the patient was on chronic therapy with clopidogrel.

**Assessment of Platelet Function**

**Aggregometry**

Platelet aggregation was evaluated by optical aggregometry in platelet-rich plasma with a Chrono-Log Lumi-Aggregometer (Probe & Go Labordiagnostica) with a constant stirring rate of 1000 rpm at 37°C as previously described.6 The final platelet count was adjusted to 300×10^3/μL with autologous platelet-poor plasma. ADP (5 or 20 μmol/L) was added, and aggregation was recorded for 5 minutes. Aggregation was expressed as the maximal percent change in light transmittance from baseline, with platelet-poor plasma as reference. Here, we report data on maximal aggregation with both ADP concentrations.

**Flow Cytometry**

The evaluation of surface expression of platelets receptors was performed in whole blood.10 The following monoclonal antibodies were used: anti-CD61 PE (anti–GP IIb/IIIa labeled with phycoerythrin; Serotec) and anti-CD62 FITC (anti–P-selectin labeled with fluorescein isothiocyanate; Beckman Coulter). In brief, citrated blood (10 μL) was diluted with 490 μL PBS and gently mixed. Both antibodies (5 μL each) and ADP or PBS (5 μL) were added to 35 μL diluted citrated blood and incubated at room temperature for 20 minutes. The samples were fixed with 300 μL of 1% buffered paraformaldehyde and then analyzed on a Becton Dickenson FACScan flow cytometer. Mean intensity of immunofluorescence was used as an index of antibody binding and receptor surface expression.

**Statistical Analysis**

Continuous data are presented as mean±SD. The Kolmogorov-Smirnov goodness-of-fit test showed that their distribution did not deviate significantly from the normal distribution. Categorical data are presented as counts or proportions. Differences between groups were assessed with Fisher’s exact test for categorical data. Paired t tests were used to compare continuous data obtained before and after loading with 600 mg clopidogrel in the same group, and unpaired t tests were used to compare continuous data between the 2 groups. Statistical significance was accepted for a 2-tailed value of P<0.05.

**Results**

The Table shows the baseline characteristics of the patients according to the presence or absence of chronic therapy with clopidogrel. There were no significant differences between the first-use group and the chronic therapy group except for the plasma concentration of C-reactive protein, which was lower in the chronic therapy group.

**Clopidogrel Loading in First-Use Patients**

Platelet aggregation induced by 5 and 20 μmol/L ADP was significantly reduced at 6 hours after administration of the loading dose of 600 mg clopidogrel compared with the preloading values in this group (P<0.001; Figure). In addition, 20 μmol/L ADP–induced expression of GP IIb/IIIa (from 738±26 to 705±42; P=0.005) and P-selectin (from 335±21 to 274±39; P<0.001) receptors on platelets was significantly reduced with 600 mg clopidogrel.

**Clopidogrel Loading in Patients With Chronic Therapy**

Loading with 600 mg clopidogrel caused intensive inhibition of ADP-induced aggregation in this group for both 5 and 20 μmol/L ADP concentrations (P<0.001; Figure). In addition, 20 μmol/L ADP–induced expression of GP IIb/IIIa (from 694±62 to 682±69; P=0.001) and P-selectin (from 284±64 to...
Moreover, this finding may provide support for the general notion that higher clopidogrel doses might be needed during chronic therapy. The fact that loading with 600 mg clopidogrel yields a substantially greater degree of platelet inhibition in patients on chronic therapy with 75 mg/d suggests that higher doses of clopidogrel are needed for patients with no treatment before percutaneous coronary intervention. Another interesting finding is that lower C-reactive protein levels are associated with the more pronounced inhibition observed with clopidogrel. In addition, the variability in platelet response to clopidogrel as shown in the Figure may be an indication that point-of-care testing of platelet inhibition may help to individualize peri-interventional antithrombotic therapy.

Study Limitations
We included in this study patients who needed an invasive procedure because of coronary artery disease. Early and sustained clopidogrel therapy has shown clinical benefit in these patients. This finding precluded the use of a more rigorous study design based on a single group of patients in whom washout periods are applied between acute loading and initiation of chronic therapy with clopidogrel. The lack of comparator control arms with 300-mg loading doses of clopidogrel is another limitation of this study. Finally, we recognize that a more pronounced platelet inhibition by antiplatelet drugs may not translate into clinical benefit and that there may be safety concerns relating to increased bleeding with higher doses of clopidogrel. Although the front loading with 600 mg did not exacerbate the bleeding hazards after percutaneous coronary interventions in a recent trial, the efficacy and safety of clopidogrel dose adjustments supported by the present study obviously need to be proved by specifically designed clinical trials.

Discussion
This study focused on the magnitude of platelet inhibition achieved with a loading dose of 600 mg clopidogrel in patients with and without chronic therapy with clopidogrel. The results may be summarized as follows. First, a 600-mg loading dose of clopidogrel given to patients not previously treated with this drug achieved a degree of platelet inhibition similar to that seen with chronic therapy of 75 mg/d. Second, a 600-mg loading dose of clopidogrel given to patients on chronic 75-mg/d therapy further suppressed platelet aggregation.

As long as a higher degree of platelet inhibition remains the goal of peri-interventional and long-term antithrombotic therapy in patients with coronary artery disease, the findings of the present study may have relevant implications with regard to the dosing of a key treatment option such as clopidogrel. The fact that loading with 600 mg clopidogrel yields a substantially greater degree of platelet inhibition in patients on chronic therapy with 75 mg/d suggests that higher clopidogrel doses might be needed during chronic therapy. Moreover, this finding may provide support for the general use of clopidogrel loading in patients undergoing percutaneous coronary interventions regardless of the presence or absence of prior chronic clopidogrel therapy. Additionally, the more pronounced inhibition observed with clopidogrel loading in patients on chronic therapy compared with patients without previous treatment with clopidogrel suggests that the degree of platelet inhibition attainable can be augmented and that a dose of clopidogrel of >600 mg could provide even more effective loading for patients with no treatment before percutaneous coronary intervention. Another interesting finding is the lower C-reactive protein level in patients on chronic clopidogrel therapy. Because there were no significant differences between the 2 study groups with regard to baseline characteristics, this finding might be attributed to the anti-inflammatory effects described for clopidogrel. In addition, the variability in platelet response to clopidogrel as shown in the Figure may be an indication that point-of-care testing of platelet inhibition may help to individualize peri-interventional antithrombotic therapy.

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Acknowledgments
The study was supported by a research grant from Deutsches Herzzentrum, Klinik an der Technischen Universität, Munich, Germany (KKF 04-03). Dr Schömig has received research grants for the Department of Cardiology from Boston Scientific, Bristol-Myers Squibb, Cordis/Johnson & Johnson, Guidant, and Lilly.

References
following percutaneous coronary intervention: a randomized controlled trial. JAMA. 2002;288:2411–2420.


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Circulation. 2004;110:1916-1919; originally published online July 19, 2004;
doi: 10.1161/01.CIR.0000137972.74120.12
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/14/1916

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